PROGRESS REPORT --
1997

CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE

P.O. Box 14565, Gainesville, FL 32604
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This report includes results of research in progress. It is not intended for publication, and should not be referred to in literature citations.

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The **Center for Medical, Agricultural and Veterinary Entomology (CMAVE)** is the first ARS-USDA research center devoted exclusively to entomology. The overall goal of the research program is to develop integrated management technologies and strategies for insects and other arthropods of agricultural, medical and veterinary importance. This report provides abstracts of research in progress and is not intended for citation in any publication. Reprints of published articles may be obtained by writing the individual authors at their CMAVE address.

MISSION STATEMENT

The Center will conduct research on insects of agricultural, medical and veterinary importance with the goal of achieving control of pest species through the development of environmentally acceptable approaches. Emphasis is placed on developing components and systems for integrated pest management, based upon an understanding of the behavior, physiology and ecology of pest species. Sensitive detection devices that employ semio-chemicals and electronic technology will provide the means for early intervention. Investigations will lead to biological control based on parasites, predators and microbes, and thus provide alternative biorational tools for managing populations of pest species. Special attention is focused on insect pests of field and horticultural crops, stored products and on arthropod pests of medical and veterinary importance. Protection of humans from arthropods of medical importance is a continuing priority. The scope of the Center's research is national and international and impacts agricultural production, postharvest storage and transport of agricultural commodities, and protection from household and disease carrying arthropods. Research is conducted to meet the needs of state and federal regulatory agencies, the Department of Defense, industry, universities, growers, commodity groups and the public at large.

STAFF AND ORGANIZATIONAL CHANGES

During the past year there have been a number of changes in the scientific staff of the CMAVE. Dr. Gary Mount retired from ARS after conducting pioneering research on insect modeling, and served for more than a decade as Director of the Medical and Veterinary Entomology Laboratory. In addition, Dr. A. Undeen recently retired from ARS, thus concluding many years of research on microsporidia, and Dr. A. Cockburn has left ARS to take a university position. At the same time, we are pleased to announce the addition of three new entomologists to our permanent scientific staff: Dr. Nancy Epsky, who is now part of the Postharvest and Bioregulation Unit, Dr. Robert Meagher, who is conducting research in the Insect Behavior and Biocontrol Unit, and Dr. Steven Valles, who works in the Imported Fire Ants and Household Insects Unit.

HONORS

Several scientists at CMAVE received significant state and national awards during 1997, as follows: Dr. Everett Mitchell received the Florida Entomological Society Entomologist-of-the-Year Award and Dr. David Williams was presented the FES Achievement Award for Research. Dr. Richard Brenner was selected as Outstanding Scientist of the year by ARS-USDA. Dr. James Tumlinson was elected to fellowship in the U.S. National Academy of Sciences.

Herbert Oberlander
Center Director

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H. Oberlander, Center Director

MISSION

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This Research Unit describes, analyzes and manipulates insect behaviors that are responsible for visual and chemical stimuli that regulate reproduction, feeding, foraging and migration. Principles of behavior are emphasized, especially reproductive behavior of pest and beneficial insects. Results of this research are applied directly to control programs and technology, such as genetic eradication programs against Mediterranean fruit flies in Central America and Caribbean fruit flies in Florida, and integrated pest management of lepidopterous pests of field and vegetable crops.

Chemistry

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This Research Unit investigates the chemical, biochemical and physiological factors that regulate insect behavior and the interaction of insects with plants and other organisms in the environment. The research program focuses on the following major areas: identification, synthesis, and behavioral evaluation of pheromones that regulate mating and other behaviors of important insect pests; identification, synthesis and behavioral evaluation of kairomones and other semiochemicals that influence the foraging behavior of beneficial entomophagous insects; identification, synthesis and behavioral evaluation of plant-produced chemicals that influence the behavior of insects; and elucidation of the biochemical mechanisms that regulate insect pheromone production, release and perception.

Imported Fire Ant and Household Insects

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This Research Unit develops reduced-risk integrated management strategies for cockroaches and their attendant allergens, pest ants, fire ants and termites. Areas of research include insecticide detoxification mechanisms; spatially-based risk assessment and insect behavioral ecology pertaining to the development of baits, repellents, and biological control agents; population dynamics; sociobiology of insects; bioecology and biodiversity; and pheromone chemistry and chemical ecology.

Mosquito and Fly

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Research in this Unit results in new technology that provides the basis for integrated management of mosquitoes and filth flies. Recipients and end-users of Unit research include livestock producers; animal, public, and vector abatement organizations; military personnel; and the public. Specific areas of research include: the biological control of mosquitoes and flies (microbial pathogens, parasites, parasitoids); the regulation of fly populations via manipulation of host attraction, host selection, and blood feeding factors; the development of personal protection technology, including the discovery of new mosquito attractants and repellents; and the discovery and use of genetic, biochemical, and physiological factors as regulating mechanisms for populations of mosquitoes and flies.

Postharvest and Bioregulation

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This Research Unit conducts research on the detection, population estimation and control of stored product insects. New detection tools are developed based on acoustical and electronic methods, as well as chemical ecology. Research approaches to population management include the application of insect behavior, molecular biology, biochemistry and tissue culture to the control of growth and development of these insects.

EXAMPLES OF RECENT RESEARCH

Parasites Smell Success

Parasites and predatory arthropods often prevent plants from being severely damaged by killing herbivores as they feed on the plants. A breakthrough in understanding how these biological control agents locate their insect hosts was achieved with the isolation and identification of a volatile chemical, "volicitin", obtained from the oral secretions of beet armyworm caterpillars. When applied to damaged leaves of corn seedlings, volicitin induced the seedlings to emit volatile compounds that attract parasitic wasps which are natural enemies of the caterpillars.

Fruit Flies Find New Trap Alluring

A trapping system based on a new 3-component lure, has been developed for the Mediterranean fruit fly. It has been tested successfully in seven foreign countries. It was also highly effective during a recent Medfly eradication program in Tampa, Florida. An improved lure and trapping system has also been developed for the Caribbean fruit fly.

Genes on the Move

The ability to insert genes into *Drosophila* suggested opportunities for a new approach to insect control. Progress in this area, however, has been held up by a lack of genetic transposons that would allow scientists to insert genes of choice in other insect species of economic importance. Recent experiments with both fruit flies and moths are showing promise. There is new evidence that a new transposon, *piggybac*, will function in both the Indianmeal Moth and the Mediterranean fruit fly, while another transposable element, *hopper,* was isolated from the Oriental fruit fly.

Taking a Bite out of Fire Ants

When Imported fire ants were introduced into the United States, almost all of their natural enemies remained behind in South America. Efforts to introduce biological control of fire ants have led to the first release in the United States of a South American phorid fly. Fly eggs hatch into larvae in the fire ants, and have the peculiar effect of decapitating the host. Then, the flies complete their development in the severed head capsule until they emerge as adult flies.

Houseflies Succumb to Worms

Adult houseflies that develop from larvae infected with parasitic nematodes lived only half as long as uninfected flies. This nematode species, originally collected from Brazil, has potential as a biological control agent for houseflies because it appears to be host specific and can be raised easily in large quantities. Moreover, because there are few natural enemies that attack flies in the larval stage this nematode may be compatible with other biological control agents.

Eavesdropping on Insects

Highly sensitive methods have been developed for detection of hidden infestations of insects in stored grain based on the sounds that are made as the larvae feed. Field trials indicate practical potential for using a sampling system with sound detectors for quantitiative sampling of hidden infestations. In addition, a commerical grain probe trap was modified by incorporating a sensor head with infrared electronics so that insects that enter the trap can be electronically sensed and counted. These detection methods may be applicable to a wide variety of insects.

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CRIS - 6615-22000-011-00D--Behavioral Ecology and Management of Crop Insect Pests with Semiochemicals

CRIS - 6615-22000-013-00D--Insect Biological Control Through Behavioral and Genetic Manipulation

CRIS - 6615-22000-014-00D--Biological Control Through Artificial Rearing of Natural Enemies and Manipulation of Host Plant Resistance

CRIS: 6615-22000-011-00D

SURVIVAL AND HOST ATTACK ACTIVITIES OF TWO SPECIES OF PARASITOIDS OF DIAMONDBACK MOTH IN FIELD CAGES DURING WINTER

G.Y. Hu, E.R. Mitchell and J.S. Okine

Objective: Determine the feasibility of colonizing two species of parasitoids in field cages to provide sources for inoculative releases in cabbage for control of the diamondback moth (DBM).

Methods: Cages were set up in a cabbage production area west of Bunnell, Flagler County, Florida, from November 1996 to February 1997. Two kinds of cages were used in this study: screened laundry hampers (61 cm high with a bottom of 38 x 27 cm and a top of 46.4 x 33.7 cm, and large screened cages (2.5 x 2.2 x 1.0 m). To initiate the test, individually potted collard plants infested with DBM larvae were placed into the large screened cages. Seventy to 75 pairs of *Diadegma insulare* (Cresson) were introduced into the two large screened cages in late November and early December, respectively, when the DBM had reached 2nd instar. *Cotesia plutellae* (Kurdjumov) was introduced into 9 laundry hampers with 1st instar DBM; three set ups were made monthly starting in November and ending in January. Also one large screened cage was set up in early December. Every two weeks, the large screened cages were monitored for larval parasitism, and individually potted collard plants infested with DBM were re-introduced. *Cotesia* cocoons in the hampers were harvested 4 weeks after set-up. Samples of cocoons were brought into the Gainesville laboratory and checked for parasitism, emergence, and sex ratios of the parasitoids.

Results: Throughout the winter, both parasitoid species attacked diamondback moth larvae, completed development within the host, and increased their numbers in the cages. Parasitism of diamondback moth larvae by *C. plutellae* was 36-42% in laundry hampers, and 35-65% in the large screened cage. Emergence of *C. plutellae* cocoons was 82.6%. The sex ratios of the emerging adults was 1:0.8-1.3. Likewise, parasitism of the diamondback moth by *D. insulare* was 55-90% in laundry hampers, and emergence from the cocoons was 89%. The sex ratio was 1:1.4-2.1 in the large screened cage. Our results showed that, in northeast Florida, it is possible to rear these diamondback moth parasitoids in field nursery cages during the winter to provide parasitoid sources for inoculative releases for DBM control in cabbage during the winter-spring cabbage season.

CRIS: 6615-22000-011-00D

RESPONSE OF *DIADEGMA INSULARE* TO CATERPILLAR FEEDING

G.Y. Hu[1], E.R. Mitchell, D. Sieglaff[1] and J.S. Okine[1]

Objective: To determine 1) If prior experience feeding on host caterpillar increases response of *D. insulare* to host and non-host infested plants, and 2) whether the naive and experienced wasps have the same response rates to host and non-host infested plants.

Methods: A flight tunnel bioassay was used to evaluate attraction responses of female *Diadegma insulare*, a parasitoid of diamondback moth (DBM), to collard plants infested with host and non-host caterpillars. Newly eclosed wasps obtained from the laboratory colony and were fed a water and honey solution. Experienced wasps were obtained by exposing them to a plant-larval complex (collard plant + DBM larvae) in a screened cage for 5-10 minutes. Mono-choice tests were conducted to test experienced and naive parasitoids for responses to the collard plants infested with diamondback moth larvae. The same design was used to compare the responses of parasitoids to plants infested with host (DBM) larvae with those infested with non-host larvae (cabbage looper, CL and cabbage worm, CW). The experiment assayed the upwind flight response of 10 naive and experienced wasps to host and non-host infested plants. Data were analyzed using two-way Anova and paired student's t-test. Percentage of responses was transformed using arsine of square root to meet the assumptions of Anova before analyses.

Results: Parasitoids with prior experience to a plant-larval complex had significantly higher ($t_{(9)}$ = 5.88, $P < 0.01$) response rates (56 ± 5.5%) when compared to inexperienced parasitoids (13 ± 3.3%). Such an increase indicates the evidence of associative learning in this parasitoid of the odor released from the damaged plants. If the wasps had no such experience when they were released into fields, they would not perform at optimum capability. Therefore, if the parasitoid has prior exposure to a plant-larval complex before release, their search efficiency for DBM will increase. In the comparison of response to host and nonhost larvae infested plants: 23.7 ± 2.7% naive *D. insulare* was attracted to DBM infested plants and 20.1 ±3.4% to CL infested plants; 49.2 ± 4.2% experienced wasps was attracted to DBM infested plants and 47.4 ± 5.4% to CL infested plants. The differences between naive and experienced wasps were significant (F = 35.9, $P < 0.01$), but were not significant between DBM and CL infested plants (F = 0.60, $P > 0.05$). Furthermore, there were no significant differences of the response between DBM infested plants and cabbage worm infested plants (F = 0.18, $P > 0.05$), but there was a significant difference between naive and experienced parasitoids (F = 59.9, $P < 0.01$). This indicates that plants damaged by host and non-host caterpillars may release common chemicals which are attractive to *D. insulare*. This may benefit an inoculative parasitoid release program because during a low host larval population, the plant odor caused by non-host larval feeding may impede the released parasitoids from leaving the field in which they were released. Furthermore, plant odors caused by generalist herbivore feeding may facilitate finding of the host by this parasitoid.

CRIS: 6615-22000-011-00D

DISCRIMINATION OF (Z)-7-DODECENYL ACETATE BY *TRICHOPLUSIA NI* (HÜBNER): EFFECT OF (Z)-7- AND/OR (Z)-9-TETRADECENYL ACETATES

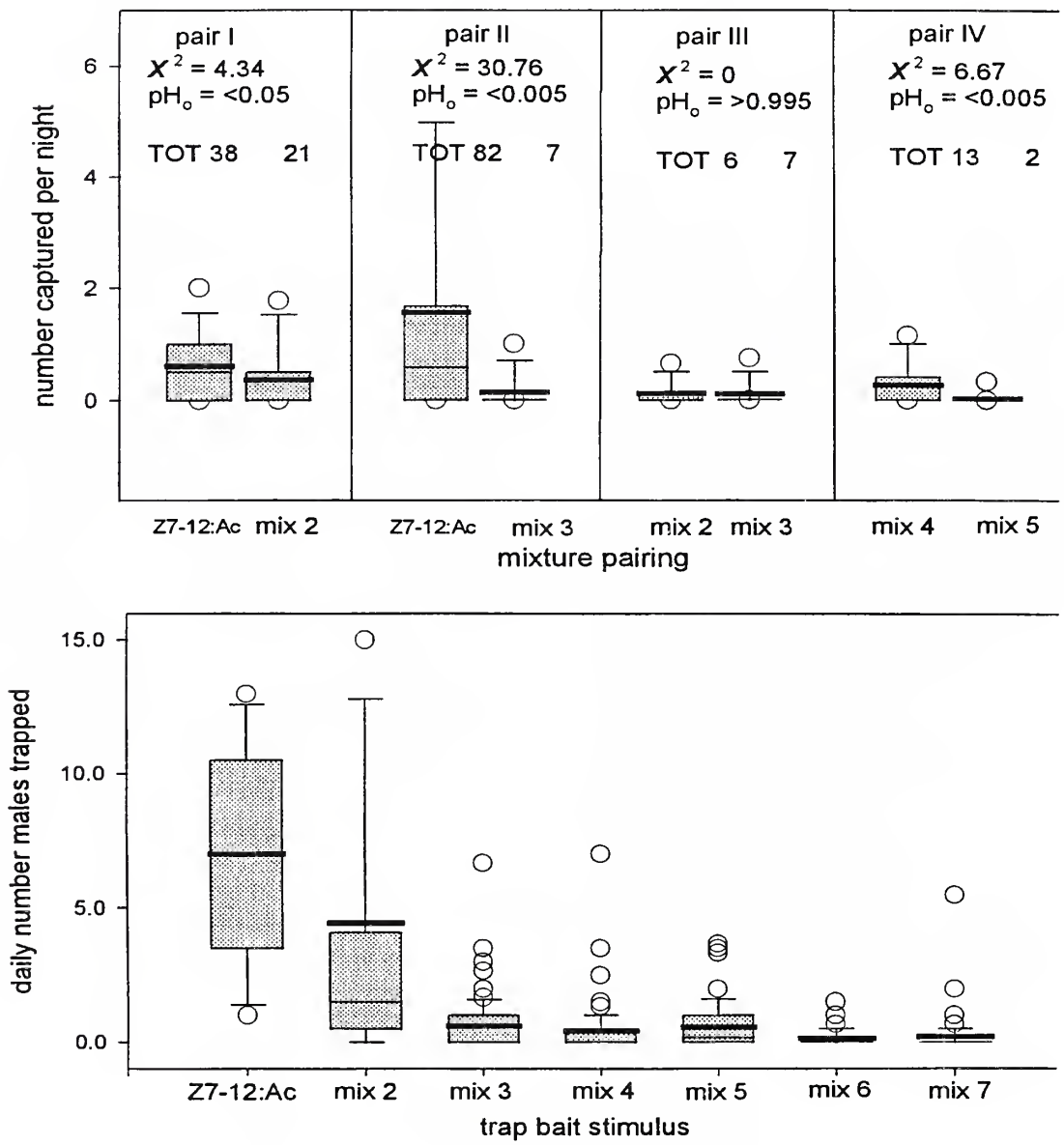
M.S. Mayer and E.R. Mitchell

Objectives: It previously was found that one or both of the two 14-carbon female sex pheromone components was responsible for male discrimination of a 6-component mixture of sex pheromone components from Z7-12:Ac. These assays were designed to determine which of the two 14-carbon acetate components of the cabbage looper pheromone is responsible for discrimination.

Methods: Two types of field trap groupings were baited with one of five mixtures of sex pheromone components and Z7-12:Ac. One grouping consisted of four paired WORT traps; the other was three lines comprised of seven unpaired traps baited with 4 of the same mixtures used in the WORT traps, Z7-12:Ac and a blank. The baits were changed every two weeks and counts of trap captures were obtained over 23 intervals ranging from 2-3 nights. The two mixtures that were the focal point of this research comprise the four 12-carbon acetates plus either Z7-14:Ac (Mix 2) or Z9-14:Ac (Mix 3). Other mixtures were assayed that tested some further details of discrimination (Mixes 4 - 6, and a blank (Mix 7).

Results: Results of the above assays are in Figure 1. The previously observed manifestation of discrimination was a decrease in the numbers of males captured at a Z7-12:Ac baited trap in a WORT paired with the six component sex pheromone mixture. The result of the WORT trap assays (figure, upper) show that the trap baited with Z7-12:Ac captured 38 males when paired with Mix 2 throughout the assay; the trap baited with Z7-12:Ac captured 82 males when paired with Mix 3. The difference between the two traps baited with Z7-12:Ac exceed the difference necessary to reject the null hypothesis that there is no difference between the two captures ($X^2 = 15.41$, $pH_o < 0.005$, 1 df). Thus, Z7-14:AC is the component which was presented in mix 2, or was absent in mix 3 and was responsible for the reduction in trap captures by Z7-12:Ac. In the unpaired trap assay, none of the mixtures captured as many males as Z7-12:Ac, and there was no difference in the number of captures by mixtures two and three. Only traps baited with Z7-12:Ac captured more males than an untreated septum (Mix 7).

Fig. 1. Field trap assays of cabbage looper sex pheromone component mixtures and Z7-12:AC.



CRIS: 6615-22000-011-00D and 6615-22000-013-00D

PHENYLACETALDEHYDE ENHANCES UPWIND FLIGHT OF MALE FALL ARMYWORM *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) TO ITS SEX PHEROMONE

R.L. Meagher, Jr. and E.R. Mitchell

Objective: The objective of these experiments was to determine if the floral compound, phenylacetaldehyde (PA), enhances the attractiveness of sex pheromones to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), in flight tunnel and field bioassays.

Methods: Three to seven-day-old, laboratory-reared males were used in the flight tunnel bioassays. Tests were conducted 1-4 hours post-scotophase in a Plexiglas box flight tunnel. Treatments consisted of a control pheromone lure and commercial pheromone lures with and without PA. The standard lure contained a ratio of (*Z*)-9-tetradecen-1-ol acetate (*Z*9-14:AC) (80.3%), (*Z*)-11-hexadecen-1-ol acetate (*Z*11-16:AC) (19.2%), and (*Z*)-7-dodecen-1-ol acetate (*Z*7-12:AC) (0.5%), loaded with 2 mg in a solvent refined rubber septum. A hexane solution of 10 mg/ml of PA was prepared, and 100 μl of this solution was pipetted onto filter paper. Moths were tested individually in the tunnel by counting the number of upwind flights and contacts with the source screen cage. Each replicate contained 5 to 20 moths, with totals of from 70 — 150 moths tested per treatment. The experiment was designed as a randomized complete block, and percentage responses were transformed into arcsine-square roots before analysis of variance.

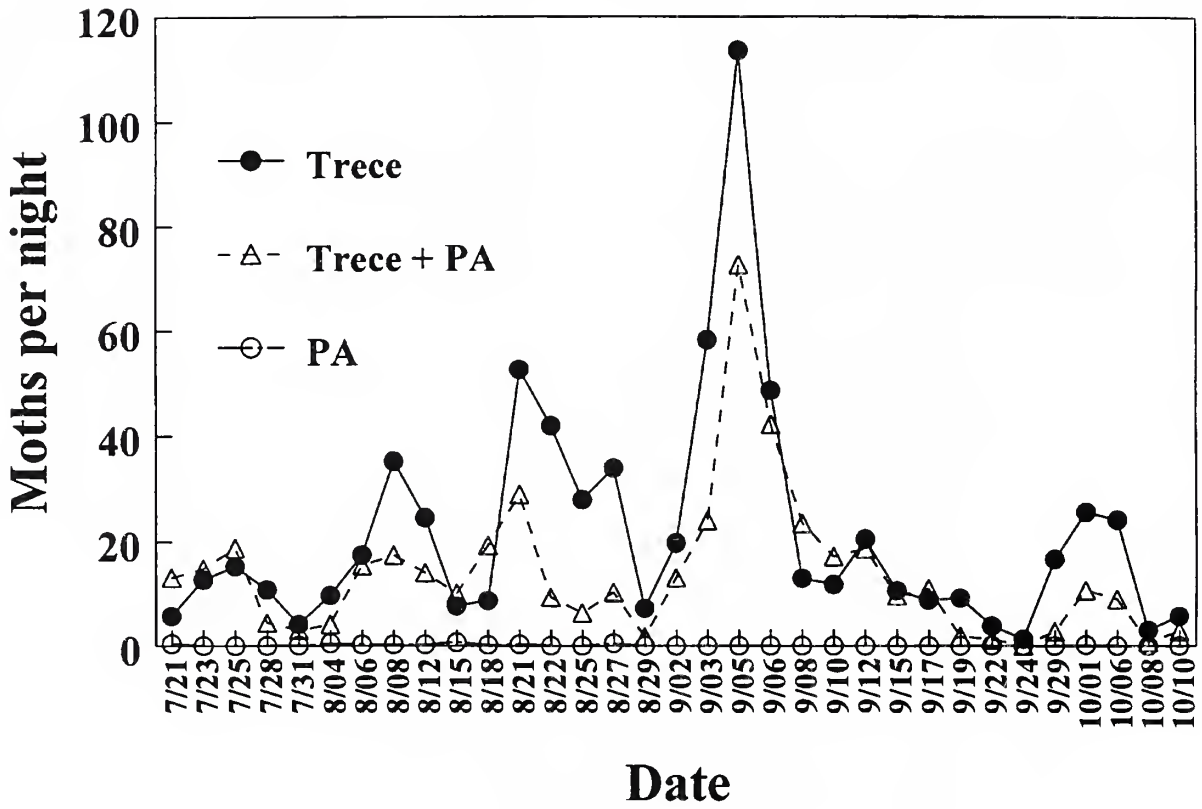
The field component of the experiment was conducted from late July to mid-October within a series of large cotton fields in Alachua, FL. Sex pheromone-baited bucket traps were set along irrigation pivots or roadways. The treatments consisted of Trécé® pheromone lure alone, Trécé lure plus a plastic vial or cap filled with 0.2 or 0.5 ml PA, or PA alone. There were three replications and trap position within each replication was changed weekly. Pheromone lures and PA caps were changed biweekly, and moth collections were made three times per week.

Results: In the flight tunnel bioassays, more males flew upwind to combinations of pheromone-treated septa and PA. Phenylacetaldehyde in combination with the laboratory-loaded lure increased upwind flight from 68.6 to 87.4%, and increased contact with the source cage from 51.9 to 76.6%. The combination of PA with a Scentry® lure increased the flight responses from 61.1 to 80.0% and contact from 30.8 to 56.3%. Phenylacetaldehyde in combination with a Trécé® lure elicited flight increases from 66.5 to 88.3% and contact from 48.7 to 75.0%. None of the moths flew upwind to PA alone at the dose tested.

In the field test, significantly more moths were collected in traps baited with a Trécé lure (mean moths per night, 21.6) than Trécé plus PA (13.7) or PA alone (0.1) (Fig. 1). The apparent positive response of moths to pheromone plus PA in the flight tunnel was not confirmed in the field trap assays. The lower numbers of moths in pheromone plus PA traps in the field shows that moths perceived PA but it did not increase the numbers of moths captured in the traps.

FAW --- Cotton

Alachua, 1997



CRIS: 6615-22000-011-00D

USING COLLARD PLANTS AS A TRAP CROP FOR DIAMONDBACK MOTH

E.R. Mitchell, G.Y. Hu[1], D. Sieglaff[1] and J.S. Okine[1]

Objective: Our previous studies showed that diamondback moth (DBM) invaded cabbage from field margins and that collard could be used as a trap crop for DBM in cabbage fields. The objective of this study was to determine if collard greens planted in field margins would impede DBM from invading the interior of cabbage fields.

Methods: Collard greens (*Brassica oleracea* var. *acephala* L.) were transplanted in the margins of three cabbage fields near Bunnell, Flagler Counry, Florida in spring 1997. Two rows of collard plants were planted along the sides of cabbage fields and seven collard plants were planted at the end of each row. The areas of fields A, B, and C were 25, 31, and 13 acres, respectively. DBM larvae and cabbage looper moth (CL) larvae were sampled on cabbage plants and collard plants weekly throughout the growing season. The raw numbers of larval counts per plant were transformed by square root (n+l) to normalize the data. The DBM counts between cabbage and collard were analyzed using a t-test on each date for each field.

Results: The results from the three fields were similar. DBM larval counts per cabbage plant remained less than 0.1 for fields A and B, and 0.15 for field C throughout the season. The larval counts on collard plants, however, increased dramatically in early March, and peaked in the middle of March with 0.8 larvae per plant for field A, 0.9 for field B and 1.1 for field C, and declined in early April. The numbers of DBM larvae on collard plants were significantly greater (P <0.05, t-test) than those on cabbage plants on March 5, 13, and 20, respectively, for all the fields. The larval counts per collard plant were higher than the threshold for pesticide spray on March 13 and 20 for field A, March 13, 20 and April 9 for field B, and March 5, 13 and 20 for field C.

When the combined numbers of DBM and CL larvae are considered on a per plant basis, the larval density per cabbage plant exceeded the threshold for pesticide spray on March 13, 20 and 27 for field A, March 13, 20, and April 2 for field B, and March 13 for field C. The results showed the collards planted in field boundaries prevented DBM from invading cabbage plants in the fields, but had no effect on CL. We conclude that provided no heavy infestation of CL occurs in the cabbage, there is no need to use pesticides when collards are planted in field margins. However, if CL infestation occurs, the farmers will need to consider using pesticides.

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CRIS 6615-22000-011-00D

EVALUATION OF PHEROMONE TRAP LURES AS MATING DISRUPTANTS FOR CORN EARWORM AND TOBACCO BUDWORM IN COTTON

E.R. Mitchell and M.S. Mayer

Objective: Although we have disrupted mating of the beet armyworm, fall armyworm, and some other species, we have had only limited success in disrupting mating of the corn earworm (CEW) and tobacco budworm (TBW) using high doses of incomplete pheromone blends. The objective of this study was to determine if pheromone blends commonly used as trap lures would disrupt mating by CEW or TBW, respectively, when deployed individually in small plots of cotton.

Methods: Pheromone lures for CEW (Hercon) and TBW (Scentry) were deployed individually in 7 x 7 arrays at the rate of 200 per acre in 0.25 plots of cotton on 15 July 1997. The treatments, including an untreated control, were set out in a N-S direction (perpendicular to the prevailing wind) in three randomized complete blocks with 208 ft between treatments and at least 1500 ft between blocks. Each plot had a wire cone trap baited with either CEW or TBW pheromone lure and a plastic tray used as a mating platform for sentinel females positioned near the center. The traps and mating platforms were set about 3 ft above ground level on metal poles.

The pheromone traps were checked for captured male moths 3-4 times per week from 18 July through 18 August. Sentinel female moths (8-10) from our laboratory colony were set out about sundown 1-2 times/week. Female moths were collected the following morning and returned to the laboratory where they were dissected to determine mating status. The pheromone traps were removed from each plot on nights sentinel female moths were set out.

Results: By using lures known to be attractive to males when used in traps, it was hoped that mating would be disrupted by the male's propensity to follow 'trails of pheromone' and thus be distracted from pheromone emitted by sentinel females located on mating platforms. However, neither the CEW nor the TBW lures were effective at preventing mating between sentinel females and wild, conspecific males.

CRIS: 6615-22000-011-00D

USE OF PHEROMONE 'SUPER SITES' TO DISRUPT MATING BY BEET ARMYWORM IN COTTON

E.R. Mitchell and M.S. Mayer

Objective: Previous research showed that application of 200 Shin-Etsu pheromone rope dispensers applied 2/plant at equally spaced intervals throughout an acre of cotton (i.e., 100 sites/ac) was highly effective at disrupting mating by beet armyworm. The objective of this study was to determine if the same number of dispensers (200) deployed in bundles of 2 to 20 ropes/site at equally spaced intervals throughout an acre of cotton would be equally effective at reducing mating by beet armyworm.

Methods: Shin-Etsu pheromone 'rope' dispensers (Lot no. 84014) were deployed at the rate of 200 units per ac in cotton on 17 July 1997 in bundles of 2, 4, 8, and 20 dispensers each in equally spaced arrays of 10 x 10, 7 x 7, 5 x 5, and 3 x 3, respectively. The dispensers were threaded individually through hardware cloth mesh attached to a wooden stake. The stake was driven into the soil to a depth that supported the dispensers about 8 inches above ground level.

The treatments, including an untreated control, were set out in a N-S direction (perpendicular to the prevailing wind) in three randomized complete blocks with 208 ft between treatments and at least 1500 ft between blocks. Each plot had a bucket trap baited with beet armyworm pheromone and a plastic tray used as a mating platform for sentinel females positioned near the center. The bucket traps and mating platforms were set about 3 ft above the ground on metal poles.

The pheromone traps were checked for captured male moths 3-4 times per week from 18 July through 30 September. Sentinel female moths (8-10) from our laboratory colony were set out about sundown 1-2 times/week. Female moths were collected the following morning and returned to the laboratory where they were dissected to determine mating status. The pheromone traps were removed from each plot on nights sentinel female moths were set out.

Results: All treatments were highly effective at preventing mating by beet armyworm for >90 days. The results show that as few as 10 pheromone 'super sites' per acre was just as effective at preventing mating by beet armyworm as 100 sites when the same quantity of pheromone was used. Very late in the season when the cotton was maturing, a few more males were captured and a few more females were mated in the 3 x 3 array than in each of the other arrays but the differences were of no consequence this late in the growing season.

Fewer pheromone sites per acre should result in greatly reduced labor costs associated with application. If a different pheromone delivery system can be found that emits the same quantity of pheromone from the reduced number of delivery sites per acre, the cost of pheromone treatment for beet armyworm conceivably would be reduced further and the technology would become even more attractive to growers.

CRIS: 6615-22000-011-00D

OVIPOSITION RESPONSE OF *COTESIA PLUELLAE* TO STERILE AND NON-STERILE DIAMONDBACK MOTH LARVAE AND SPATIAL DISPERSION OF LARVAE ON COLLARD PLANTS

J.S. Okine, E.R. Mitchell, J. Carpenter and G. Y. Hu

Objective: To assess the suitability of sterile diamondback moth (*Plutella xylostella*) larvae as host for *Cotesia plutellae*.

Methods: Two sets of potted collard (*Brassica oleracea var acephala* (L.)) plants were each infested with 20 second instar sterile and normal diamondback (*Plutella xylostella* (L.) (DBM) larvae respectively and set in an outdoor screened field cage (2.7 X 2.3 m). Twenty-five mating pairs of *Cotesia plutellae* were introduced into the cage. The parasitoids were allowed 48 h to sting the DBM larvae. After that the larvae were collected and reared in the laboratory until pupation of the DBM larvae or *C. plutellae,* mortality and proportion of DBM parasitized was recorded and calculated. *Cotesia plutellae* pupae from the larvae were held for emergence and percentage emergence calculated. Foliage consumption between the two sets of larvae for the period of parasitoid exposure was also measured.

Results: There was no significant difference in percent parasitism and mortality between the sterile and normal larvae. Percent emergence and larval foliage consumption did not differ significantly between the two sets of larvae. Our results suggest that sterile larvae could serve as appropriate hosts *for C. plutellae*.

CRIS 6615-22000-011-00D

REARING FIELD-TRIAL QUANTITIES OF *DIADEGMA INSULARE*, AN ENDOPARASITOID OF DBM

D.H. Sieglaff[1], E.R. Mitchell and G.Y. Hu[1]

Objective: To determine an effective and economically feasible method to produce field-trial quantities of *Diadegma insulare* (Cresson).

Methods: *D. insulare* were originally collected from collards adjacent to cabbage fields near Bunnell, Flagler County, Florida, May 1996, and have been reared since on laboratory reared *Plutella xylostella* (L.), diamondback moth (DBM). Both host and parasitoid colonies were maintained in rearing rooms at 25°C, 50% RH and a photo period of 14:10 (L:D) h, DBM reared on a wheat germ-based artificial diet, and *D. insulare* on DBM inoculated collard leaves. Artificial diet cakes and rearing cups were impregnated with collard extract in an attempt to stimulate parasitism of DBM feeding on artificial diet cakes. Artificial diet containing 10 and 23% collard extract and 16 oz. paper cups impregnated with collard extract were compared against artificial diet without collard extract and a collard leaf control in the production of *D. insulare* adults. DBM infested diet cakes and DBM infested collard leaves (= collard leaf control) were exposed to a DBM : *D. insulare* ratio of 50:1 for 24 h and 48 h. The 24 h treatments were conducted within 5 liter plastic rearing containers, whereas collard extract impregnated diet cups were exposed to parasitism for 48 h. The number of *D. insulare* adults produced (square root transformed) and percent parasitism (√arcsine transformed)were analyzed using ANOVA, and the means separated by Duncan's multiple range test.

Results: The collard leaf control (CLC), on average, produced more *D. insulare* adults and had a higher percentage *D. insulare* emergence than all other treatments exposed to parasitism for 24 h ($P < 0.05$). The CLC produced 32 ± 3.8 (SE) adult *D. insulare* per ♀ *D. insulare* used, and had 77.2 ± 4.5 percentage parasitism. All other treatments exposed to parasitism for 24 h produced 50+% less adult *D. insulare*, and had 50+% less percentage parasitism than the CLC. However, 48 h of exposure to parasitism in the collard extract impregnated diet cups (CEIDC) produced 19.7 ± 4.3 adult *D. insulare* which was not significantly lower than the CLC ($P > 0.05$). On the other hand, 48 h of exposure in an untreated diet cup produced only 11.6 ± 2.2 adult *D. insulare*, which was significantly lower than the CLC ($P < 0.05$). This suggests that a longer exposure period coupled with the use of collard extract may enhance *D. insulare* production.

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CRIS: 6615-22000-013-00D

ISOLATION OF A NEW hopper ELEMENT FROM THE *BACTROCERA DORSALIS* white-eye STRAIN

A.M. Handler and S.P. Gomez[1]

Objective: To isolate a functional hopper element from the oriental fruit fly for use in gene transfer of tephritid fruit flies.

Methods: Sequence data from the original hopper element, which was isolated as a genomic clone from the *B. dorsalis* Kahuku wild strain, was used to create primers for inverse PCR to isolate additional hopper elements from other *B. dorsalis* strains. A new element, isolated from the white-eye strain, was identified initially by differing chromosomal insertion site sequences. Primers to 5' and 3' insertion site sequence and internal hopper sequence were used in PCR experiments to isolate the complete element.

Results: The original Kahuku 3120 bp hopper element is apparently complete, but possibly nonfunctional due to several discontinuous reading frames, and a lack of an 8 bp direct repeat at its chromosomal insertion site. A new 3131 bp hopper element was isolated by inverse and direct PCR from white-eye strain genomic DNA. This sequence contains a continuous 1.9 kb open reading frame, upstream CAAT and TATA sequences, and a 3' AATAAA poiyadenylation signal sequence, all of which is consistent with a functional transposase . In addition, this hopper has the same inverted terminal repeats as the original, but has 8 bp direct repeats in the adjacent chromosomal insertion site. As with the Kahuku hopper, BLAST comparisons show greatest similarity between hopper and Ac, hobo, and Hermes, but these relationships remain distant. Though apparently a member of the hAT transposable element family, the hopper element is the most divergent of the insect elements. If the white-eye hopper element is found to be functional based on transient mobility assays, it will be tested for gene transfer vector function in the oriental fruit fly, as well as other tephritid species.

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CRIS: 6615-22000-013-00D

piggyBac-MEDIATED GERMLINE TRANSFORMATION OF THE MEDITERRANEAN FRUIT FLY

A.M. Handler and S.D. McCombs[1]

Objective: To test the ability of the *Trichoplusia ni* piggyBac transposon vector to mediate germline transformation in the Mediterranean fruit fly, *Ceratitis capitata*.

Methods: The availability of the medfly white+ marker (kindly provided by L. Zwiebel, EMBL, Heidelberg) made it possible to test potential vector systems in medfly white-eye host strains using an unambiguous visible marker. A piggyBac element (kindly provided by M. Fraser, Univ. Notre Dame) was tested by first inserting the hsp70-regulated white+ gene, as a NotI fragment, into a NotI site created by linkers to the unique HpaI site in the piggyBac open reading frame. A piggyBac transposase helper (normally regulated) was made non-mobile by a SacI deletion of the 5' terminal sequence. Medfly white-eye host strain embryos were injected with a 500:150 μg/ml vector:helper mixture. G0 adults were outcrossed and putative G1 transformants were selected by eye pigmentation. Subsequent generations were tested for vector integrations by Southern DNA hybridization and inverse PCR.

Results: Of 800 injected embryos, 73 surviving G0s were individually outcrossed to white-eye flies, of which 19 lines were fertile. One line (Cc[pBw]41) gave rise to three G1 offspring having dark-orange colored eyes. Southern analysis of G2 and G8 tranformants using a 1.4 kb piggyBac probe (SphI-HpaI fragment) on BglII and SalI cut genomic DNA verified the presence of the vector. In all three lines BglII digestion revealed the internal 1.6 kb fragment, and a 4.5 kb fragment representing the 5' vector arm (0.7 kb) and adjacent chromosomal DNA. SalI digestion was also consistent with the same chromosomal integration in all three lines. Additional bands were detected in the G2 M1 line and the G8 F2 line, suggesting additional integrations, probably not associated with the visible phenotype. The integration was determined to be a piggyBac-mediated event by sequencing the insertion site junctions isolated by inverse PCR. A TTAA duplication of the insertion site was observed, diagnostic of all piggyBac insertions. A transformation frequency of 5-10% per fertile G0 is deduced, which is somewhat lower, but still in the range of typical Drosophila P or hobo transformation. Additional experiments are necessary to optimize the system, and to determine a reliable frequency of transformation. This experiment demonstrates, unambiguously, piggyBac vector function in dipteran species, which suggests that piggyBac will be similarly active in other dipteran species.

[1]Department of Entomology, University of Hawaii

CRIS: 6615-22000-013-00D

AIR SAMPLING FROM FIELD PLOTS TREATED WITH (Z9, E12)-TETRADECADIENYL ACETATE AND (Z)-9-TETRADECENOL TO DISRUPT MATING BY THE BEET ARMYWORM, *SPODOPTERA EXIGUA* (HÜBNER)

E.R. Mitchell and M.S. Mayer

Objective: To measure the airborne concentration of (Z9, E12)-tetradecadienyl acetate (ZETA) and (Z)-9-tetradecadienol (Z9-14:OH) within field plots that were treated with varying amounts of Shin-Etsu commercial sex pheromone dispenser ropes to disrupt mating by beet armyworm.

Methods: One acre plots in the middle of two cotton fields were treated with four different densities of Shin-Etsu commercial beet armyworm disruptant ropes. The densities were 3 X 3, 5 X 5, 7 X 7 and 10 X 10 and an untreated control plot. Each density represented a total of 200 total dispensers/ac. During the growing season, populations were monitored by sex pheromone baited bucket traps and mating disruption by mating tables containing live females. Airborne concentrations of the above two sex pheromones were measured from air samples collected for 12 hr from 2000 hr to 0800 on Super Q® filters at an airflow rate of 600 ml / min from the middle of the treated plots and from an untreated field distant from the treated fields. The adsorbed pheromones and other volatiles were eluted from the filter with methylene chloride and the samples were analyzed by glc without further preparation. At least two samples were obtained from each plot and the control field during the late part of the cotton growing season of 1997.

Results: No measurable glc peaks having retention times the same as ZETA or Z9-14:OH were observed from the control field. Peaks having the same retention time as ZETA and Z9-14:OH were observed in the air samples from all treated plots. The amounts of ZETA ranged from a high of 2.7 ng / hr (3×10^{-13} M) from one of the 3 X 3 plots to a low of 0.03 ng / hr (3.3×10^{-15} M) from a 10 X 10 plot; the amounts of Z9-14:OH ranged from 4.3 ng / hr (6×10^{-13} M) from a 3 X 3 plot to 0.40 ng / hr (5×10^{-14} M) from a 10 X 10 plot. These measures result in an airborne ratio of ZETA : Z9-14:OH ranging about 1 : 2. The emission concentration of ZETA and Z9-14:OH from a single female in an airflow of 800 m / hr (0.5 mph) can be estimated to be about 6×10^{-12} M and 2×10^{-13} M, respectively, for a ratio of 30 : 1. Of course, all these measures of airborne concentration vary widely dependent upon ambient temperature and wind conditions.

CRIS: 6615-22000-013-00D

RELATIONSHIP BETWEEN PHEROMONE COMPONENT EMISSION FROM COMMERCIAL BAITS AND CAPTURES OF *HELICOVERPA ZEA* AND *HELIOTHIS VIRESCENS* IN BUCKET AND CONE TRAPS

E.R. Mitchell and M.S. Mayer

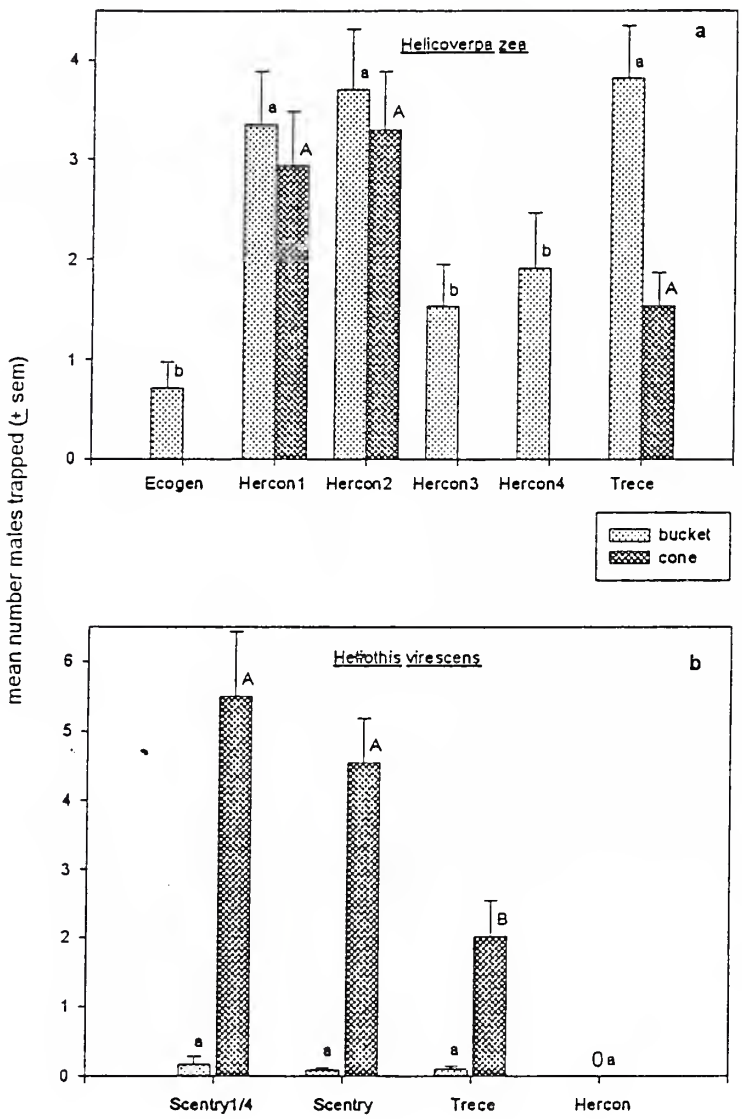
Objective: The emission of sex pheromone components from various commercial trap baits were sampled to determine why some baits effect captures of corn earworm moths, *Helicoverpa zea* (Boddie), and tobacco budworm moths, *Heliothis virescens* (F.), in bucket traps and some do not.

Methods: Two types of traps, bucket and cone, were set out in a cotton field and were baited with three commercial baits optimized to capture corn earworms and three optimized to capture tobacco budworms. Some of the baits were cut into sections to reduce the amount of emitted stimulant. The numbers of moths captured in each type of trap were counted over ten time intervals comprised of 2 or 3 days. The emission of sex pheromone components from all of the baits were collected on Super Q® filters. Compound identities were assigned by congruence of retention times with those of authentic standards. The quantity of the emitted components was determined by commonly used gas-liquid chromatographic methods.

Trap results: Both cone and bucket traps baited with three commercial baits from three manufacturers captured significant numbers of corn earworm moths (Figure 1, a). Bucket traps baited with Ecogen® baits captured statistically fewer males than the other two baits. Male tobacco budworms were only captured in cone traps (Figure 1, b). Cone traps baited with quartered and entire Scentry® baits captured statistically indistinguishable numbers of males, but both of these captured more males than traps with Trécé® baits. Neither type of trap with the Hercon baits captured male tobacco budworms. **Bait emissions:** The emission rates of the active sex pheromone components failed to explain fully the observed differences in trap captures, although some correlations seemed meaningful.

The most important observation is that the commercial Ecogen bait effected captures of corn earworms in bucket traps. Notwithstanding some of the apparent differences in captures, for the most part we were unable to decide clearly and conclusively what differences in the bait emissions determined whether or not one or the other trap would capture males of either species.

Fig. 1. Captures of *Helicoverpa zea* and *Heliothis virescens* in two types of trap using different commerical baits.



CRIS: 6615-22000-013-00D

ECOLOGY AND BEHAVIOR OF TEPHRITID FRUIT FLY PARASITOIDS IN MEXICO AND FLORIDA

J. Sivinski, M. Aluja[1] and A. Eitam

Objective: Parasitoids, including those that attack tephritid fruit flies, are often specialized. That is, they are active in certain host trees, locations, seasons, and times of day. Information on the foraging behaviors and spatial / temporal distributions of parasitoids may predict which natural enemy introductions or augmentations will be best suited to a particular circumstance. The relevant scale of distribution ranges from within tree canopies to across regions.

Methods: In Veracruz State, Mexico the spatial and temporal distributions of eight species of parasitic Hymenoptera attacking five species of *Anastrepha* fruit flies have been studied for a period of more than four years. Samples have been taken to determine within-tree canopy distributions, altitudinal distributions, and regional differences in distribution (studies have recently expanded to the state of Yucatan). In Florida, the distributions of three parasitoids across latitude have been determined and the underlying environmental causes of distributions examined. In addition field and laboratory studies of diapause have been carried out and the environmental factors associated with entry into diapause have been identified.

Results: On the scale of within-tree canopies, niche separation among native parasitoids is often more pronounced than between native and introduced species. Certain species have relatively narrower host ranges and are specialized on particular sizes of fruits. Fruit size, both within and among tree species is an important factor in parasitism, presumably because of the increased vulnerability of fly larvae in small fruits. There are distinct altitudinal differences in parasitoid distributions. These appear to be due to different tolerances for high and low temperatures. Species that live in highly variable environments are more likely to exhibit diapause and substantial portions of these populations may diapause for up to one year. As a consequence, accurate determinations of parasitism through samples of field collected insects must take parasitoid diapuase into consideration. Latitudinal distributions of parasitoids in Florida suggest that host tree diversity is an important factor in a species ability to maintain itself. That is, in the absence of diapause a variety of trees containing fruit fly hosts must be available through out the year for nondiapausing or less long lived species to compete with long-lived or diapausing species. The conservation and augmentation of native trees that preserve or enhance parasitoid populations is under consideration.

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CRIS: 6615-22000-013-00D

EFFECTS OF THE INSECTICIDE, SUREDYE, ON BENEFICIAL INSECTS WITHIN CITRUS GROVES

J. Sivinski and T. Holler[1]

Objective: Malathion bait sprays are a major means tephritid fruit fly control. However, the chronic use of Malathion is often controversial, and the chemical has been banned from certain critical locations. Suredye, a food and cosmetic additive that is toxic to insects but much less so to vertebrates, has been proposed as a substitute for Malathion in bait sprays. Unlike Malathion, Suredye must be consumed in order to take effect. A danger associated with insecticide use is the mortality inflicted on nonmarket organisms that may themselves be important in the control of pest species. Before the widespread adoption of Suredye it is necessary to determine the relative effects of Malathion and Suredye on insect natural enemies. One relevant comparison is changes in the predator and parasitoid faunas of citrus groves following treatment by the two compounds.

Methods: Blocks in a central Florida orange grove where treated with either a Malathion or Suredye bait spray or left untreated. Passive traps (i.e., those without attractants and whose ability to capture insects would not be influenced by the baits prays themselves) were deployed and monitored weekly. These consisted of Yellow-Sticky-panels and Malaise traps. In an adjacent area, McPhail traps baited with the different attractive baits used in the two types of sprays were monitored weekly to examine the attraction of nonmarket insects to the sprays. On two occasions mass releases of a fruit fly parasitoid were made in the various plots and the survival of the parasitoids monitored through the use of oviposition devices placed near release sites. In the laboratory, representative species of beneficial insects were caged with leaves removed from treated and untreated plots and their mortality was monitored over a period of 48 hours.

Results: Preliminary analysis finds Suredye bait sprays to be more benign toward beneficial insects than Malathion sprays.

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AERIAL RELEASES OF MEDFLY PARASITOIDS

J. Sivinski, T. Holler[1] and F. Jeronimo[2]

Objective: The Mediterranean fruit fly, *Ceratitis capitata*, is abundant in Central America. It is prevented from moving Northward by a barrier erected by the international organization MOSCAMED along the Mexican / Guatemalan border. Suppression of pest populations in the barrier zone is largely through the release of sterile male flies. Previous studies have demonstrated that sterile releases can be made more effective by the additional release of parasitoids. In the past, such parasitoid releases have been made from the ground. However, the rugged terrain of the Guatemalan highlands requires the development of aerial release techniques.

Methods: Sterile male Medfly fruit flies and the braconid, *Diachasmimorpha tryoni,* were released from an airplane over a mountainous area in Guatemala near the Mexican border. Parasitoids were chilled, placed in paper bags, and dropped from an altitude of ~ 100 m and at an airspeed of ~ 130 km /hr. Sterile male Medflies were released in the typical manner, i.e., chilled and dropped directly into the air. The sterile flies were also released into a second site without the addition of parasitoids. A third site remained untreated and served as a control.

Results: Egg sterility was typically less than 20% in both sterile male alone and sterile male and parasitoid sites. Parasitism was discovered only in the parasitoid release site and reached levels as high as 84%. The numbers of adult flies trapped and larvae collected from fruits were typically higher in the untreated site. During the peak of pest population growth, adult flies were lowest in the combined parasitoid and sterile fly release site.

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IMPORTATION OF MEDFLY NATURAL ENEMIES INTO GUATEMALA

J. Sivinski, T. Holler[1] and F. Jeronimo[2]

Objective: The Mediterranean fruit fly, *Ceratitis capitata*, infests over 300 species of fruits and vegetables, and because of quarantines threatens agricultural export markets throughout the tropics and subtropics. The pest is abundant in Central America, and for nearly 20 years the international organization MOSCAMED has maintained a barrier along the Mexican/ Guatemalan border to prevent its northward spread. This barrier has consisted largely of insecticide-bait sprays and sterile male releases. Additional suppression of pest populations with natural enemies would increase the efficiency of these and any other methods that might be applied in the future. At present, parasitism of Medfly in Central America is typically low and sporadic. New species of parasitoids released to attack this exotic pest might provide further control.

Methods: Candidate parasitoids are imported into Guatemala and colonized at a USDA-APHIS / MOSCAMED rearing facility. There they are examined for host range, ability to develop in irradiated hosts (an advantage in a rearing program), and propensity to compete with established parasitoids. Field cage tests in various locations determine preferred environmental conditions.

Results: Candidate species presently colonized and being considered for release are: *Diachasmimorpha krausii* (originally from Australia), *Coptera lopezi* (originally from Mexico), and *Biosteres arisanus* (from a colony in Hawaii). Explorations are underway in Africa (with the collaboration of ICIPE in Kenya) to locate further candidate parasitoids.

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CRIS: 6615-22000-014-00D

BIOLOGICALLY BASED PEST MANAGEMENT THROUGH ARTIFICIAL REARING OF NATURAL ENEMIES AND MANIPULATION OF HOST PLANT RESISTANCE

S.M. Ferkovich, H. Oberlander, J. Carpenter[1] and P. Greany

Objective: To investigate the potential of providing requisite host factors or their products in suboptimal diets for parasitoids or predators through the use of insect cell lines and/or their products..

Methods: To assess the effect of supplementing an artificial diet of a pupal parasitoid, *Diapetimorpha introita,* a modified Grace-Yunker's medium was conditioned with a cell line (IPL-LdFB) originally derived from fat body of the gypsy moth, *Lymantria dispar*. Conditioned media was then added to an artificial diet and encapsulated in paraffin domes. Two additional control diets included in the experiments were unconditioned medium with fetal bovine serum and unconditioned medium with out the serum. Newly oviposited eggs of *D. introita* were then placed on the encapsulated diet and the growth and development of the parasitoid were monitored.

Results: Cocoon production was highest on the cell-supplemented diet relative to the other treatment diets; however, development took longer than on the natural host (Table 1). The average weight of parasitoids grown on the cell line-supplemented diet and the diet prepared with unconditioned medium with fetal bovine serum was greater than parasitoids on the control diet and was comparable to the weight of parasitoids reared on the natural host, *Spodoptera frugiperida* (Table 2). Additionally, ovaries of females from larvae fed on the cell conditioned diet were more normal in appearance than those of females from larvae fed on the other treatment diets. These results suggest that the fecundity of the females may have improved and that this diet should be the focus of future research. The lower percentage of adult emergence on the cell-supplemented diet will also be addressed.

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TABLE 1. Developmental time and cocoon production by *Diapetamorpha introita* reared on different artificial diets and pupae of the natural host, *S. frugiperida*.

Treatment diets[a]	Mean Developmental Time (days)±SE		Mean % Cocoon Produced ±SE
	male	female	
Artificial diet	20.2 ±0.7	21.1 ±1.7	68.1 ±11.1
GC	19.5 ±0.2	24.7 ±1.2	67.8 ±10.1
G+FBS	20.6 ±0.5	22.1 ±1.0	69.9 ±12.9
GCellCond	18.7 ±0.4	23.0 ±1.1	83.8 ±12.5
Host Pupae[b]	16.4 ±0.3	16.2 ±0.3	N.D.
F-ratio(P-value)	1.35 (0.2972)	1.41 (0.2855)	1.86 (0.1767)

[a]Artificial diet, refers to control diet prepared with unconditioned SF 900-II medium; GC, diet prepared with unconditioned Grace's medium; G + FBS, diet prepared with unconditioned Grace's medium plus 10% FBS; and GcellCond, diet prepared with cell line-conditioned (IPL-LdFB) Grace's medium plus 10% FBS.
[b]Data for parasitoids reared on the natural host pupae were not included with other treatments in ANOVA. There was no significant difference among treatment means at the 5% level.
N.D., data not available.

TABLE 2. Adult emergence and weight of *Diapetamorpha introita* reared on different artificial diets and pupae of the natural host, *S. frugiperida*.

Treatment[a]	Mean % emergence ±SE	Mean adult wt. (mg) ±SE	
		male	female
Artificial diet	51.0 ±10.9	20.2 ±1.3[a]	31.2 ±0.9[a]
GC	43.9 ±7.7	25.8 ±2.4[b]	36.0 ±4.5[b]
G+FBS	45.0 ±7.3	26.9 ±2.2[b]	41.6 ±6.2[b]
GCellCond	36.5 ±6.5	27.2 ±2.2[b]	43.2 ±4.9[b]
Host	ND	23.7 ±0.5 (n=30)	45.2 ±1.2 (n=30)
F-ratio (P-value)	0.89 (0.4657)	2.28 (0.1244)	2.29 (0.1759)

[a]Artificial diet, refers to control diet prepared with unconditioned SF 900-II medium; GC, diet prepared with unconditioned Grace's medium; G + FBS, diet prepared with unconditioned Grace's medium plus 10% FBS; and GcellCond, diet prepared with cell line-conditioned (IPL-LdFB) Grace's medium plus 10% FBS.
[b]Data for parasitoids reared on natural host pupae were not included with other treatments in ANOVA; it is not used in Duncan's comparison of treatment means. Means with in the same column with the same letter are not significantly different at the 5% level.
N.D., Data not available.

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BIOCONTROL THROUGH ARTIFICIAL REARING OF NATURAL ENEMIES AND MANIPULATION OF HOST PLANT RESISTANCE

P. Greany

Objectives: The overall objective of this research is to develop new and improved technologies to provide ecologically-based alternatives to insecticidal control of insect pests of field and fruit crops. The primary goal is to develop low cost, effective methods to mass rear high quality beneficial insects (parasitoids and predators) that attack pest insects, so that they can be used in augmentative field releases.

Methods: Studies are being conducted on the behavior and nutritional requirements of *Podisus maculiventris* and other selected beneficial insects, and we are using this information to develop artificial rearing media and presentation systems that should dramatically lower the cost of production. Novel diet encapsulation methods are being developed with a CRADA partner's assistance toward enabling cost-effective scaleup of the process. We will evaluate the performance of the artificially-reared beneficial insects, and will integrate their use with other compatible strategies being developed by collaborators.

Results: A diet ("DI diet") we developed earlier for an ectoparasitic wasp (*Diapetimorpha introita*), and for which a patent was recently granted, was employed for rearing of *Podisus maculiventris*. Immature growth and development progressed at a rate essentially equivalent to that on natural prey (larvae of the greater wax moth, *Galleria mellonella*), but the fecundity of the resulting adult females is significantly lower (ca. 5x) than that of females reared on *G. mellonella* larvae. Tests have been initiated on incorporation of lipid extracts of *G. mellonella* larvae into artificial diets, but the results have not yet been obtained.

Success has already been obtained in rearing a gregarious ectoparasitoid of the codling moth on DI diet. Cooperative tests involving a diet developed by Dr. Guadalupe Rojas (USDA ARS Beneficial Insects Research Unit in Weslaco, TX) for a parasitoid of the boll weevil are showing promise for use of this diet as well. These tests are being conducted in cooperation with Dr. Tom Unruh of the USDA ARS Fruit and Vegetable Insects Research Lab in Wapato, WA.

Development of a polymeric coating and encapsulation process for the diet, with Analytical Research Systems, Inc., has progressed rapidly. Small diet capsules (ca. 2-4 mm diam.) are being produced routinely. However, acceptance of these capsules for feeding by *Podisus maculiventris* nymphs and adults was not noted, perhaps because of mechanical properties of the capsule shell, or because of a need to incorporate prey-recognition kairomones.

CHEMISTRY

CRIS - 6615-22000-012-00D--Chemistry and Biochemistry of Insect Behavior, Physiology, and Ecology

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ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *MANDUCA SEXTA* ORAL SECRETIONS

H.T. Alborn, M.M. Brennan and J.H. Tumlinson

Objective: To isolate and identify the substance(s) in the oral secretions of *Manduca sexta* caterpillars that induces plants to biosynthesize and release volatile compounds.

Methods: *Manduca sexta* caterpillars were collected from tobacco fields in Alachula County, FL, in August of 1996 and 1997. Oral secretion was obtained from these caterpillars by gently squeezing the caterpillars and collecting the oral secretion in a vial. Oral secretion is stored at -70°C. Crude oral secretion is centrifuged at 14,000*g* for 10 min to remove solids and the supernatant is then filtered through a 0.22 µm sterilizing membrane. Active material can be extracted from an acidified aqueous filtered supernatant with methylene chloride. Separation and purification of the active compound(s) is achieved by reverse phase HPLC on a C_{18} column. A bioassay which consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings is used to monitor fractionation of the oral secretion. An amount of each fraction equal to 15 µl of crude oral secretion is added to 500 µl of 50 mM, pH 8, phosphate buffer. A 9- to 10-day-old corn seedling is cut off above the root with a razor blade and the cut end immersed in the buffer solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over a period of 12 hr in complete darkness. Then 3 seedlings for each treatment are combined in a glass volatile collection apparatus (15 cm long, 3 cm id) under artificial light. Purified, humidified air is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. Then the adsorbent is extracted with 170 µl of methylene chloride and the extract analyzed by capillary GC.

Results: The crude oral secretion of *M. sexta* caterpillars induces corn seedlings to produce and release the same volatile compounds in similar proportions, but in much smaller quantities, than the oral secretion of *Spodoptera exigua* caterpillars. About 100 µl of *M. sexta* oral secretion is required to elicit the same response as 15 µl of *S. exigua* secretion. Activity can be extracted from an acidified aqueous solution into methylene chloride, indicating both lipid character and an acidic functional group. Thus, the structure of this substance may be similar to that of volicitin, **1**, obtained from *S. exigua* oral secretion. However, the lower activity in corn seedling bioassays and different retention characteristics on HPLC indicate that there are significant differences in the structures.

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SOURCE OF PLANT VOLATILE ELICITORS FOUND IN *Schistocerca americana* ORAL SECRETIONS

M. Donohue, P.E.A. Teal and J.H. Tumlinson

Objective: To determine which tissues in *Schistocerca americana* produce and/or store the substance(s) that induces plants to biosynthesize and release volatile compounds.

Methods: *Schistocerca americana* nymphs and adults obtained from Dr. John Capinera, Dept. of Entomology and Nematology, University of Florida, were maintained under a 14:10 LD cycle at 60 % RH and 25 °C and fed corn seedlings. Oral secretion was collected from the grasshoppers by gently squeezing them and drawing the oral secretion into a capillary pipette under a slight vacuum.

Oral secretion is stored at -70°C. Tissues were dissected from anesthetized grasshoppers under physiological saline. Hemolymph was collected in capillary pipettes by bleeding the animals after removal of the metathorasic leg. The fore- mid- and hindguts were isolated by application of ligatures prior to removal. Other tissues including the salivary glands, thorasic muscles and reproductive tissues were also removed. Tissues were homogenized in saline and the homogenates centrifuged and filtered through an sterile 0.25 µm filter. A bioassay which consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings is used to monitor fractionation of the oral secretion. An amount of each fraction equal to 30 µl of crude oral secretion or 0.25 insect equivalents of each tissue extract is added to 500 µl of saline. A 9- to 10-day-old corn seedling is cut off above the root with a razor blade and the cut end immersed in the saline solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over a period of 12 hr in complete darkness. Then the seedling for each treatment is placed in a glass volatile collection apparatus (15 cm long, 3 cm id) under artificial light. Purified, humidified air is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. Then the adsorbent is extracted with 150 µl of methylene chloride and the extract analyzed by capillary GC.

Results: The crude oral secretion of *S. americana* induces corn seedlings to produce and release the same volatile compounds in similar proportions, but in approximately 6-fold larger quantities, than the oral secretion of *Spodoptera exigua* caterpillars. When extracts of various tissues of adult male grasshoppers were bioassayed the majority of the active material was found in the foregut, with lesser amounts in midgut and hindgut extracts. No activity was found in salivary glands, hemolymph, muscles or male reproductive tract. Extracts of female foreguts and midguts were also more active than extracts of other tissues. These preliminary results indicate that the plant volatile elicitor in grasshoppers is not produced in the salivary glands. It is probably produced in the foregut. Further experiments will be conducted to determine the effect of age, maturity and physiological state on the production of the elicitor in grasshoppers.

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COTTON VOLATILES SYNTHESIZED AND RELEASED DISTAL TO THE SITE OF INSECT DAMAGE

P.W. Paré and J.H. Tumlinson

Objective: To determine whether volatiles released from cotton systemically are synthesized *de novo* in the undamaged leaves from which they are released, or in the damaged leaves and transported to the undamaged leaves.

Methods: Six-week-old cotton plants (*Gossypium hirsutum* L., var. Deltapine Acala 90) grown from seeds in an insect-free greenhouse were used in labeling studies. Beet armyworms (*Spodoptera exigua* Hübner) were reared on artificial diet in our laboratory and fourth-instar caterpillars were starved for 7 hours prior to being placed on plants. Two larvae per leaf were caged on the bottom two leaves and on one of the cotyledons at the start of the experiment (1200 hours, day 1) and were allowed to feed continuously throughout the experiment. Volatile chemicals were collected in the greenhouse from intact cotton plants on the third day. Three-hour volatile samples were taken at times when partially damaged plants emitted a maximum of volatiles (0900 to 1200, 1200 to 1500 and 1500 to 1800 hours). Volatiles were collected from the undamaged upper leaves while caterpillars fed on the lower leaves of the same plant. Labeled carbon dioxide air was introduced into the chambers at 0900 hours on day 3 for a 9-hour period and volatiles were collected in 3-hour intervals. Compounds were quantified via flame ionization detection and the amount of ^{13}C incorporated into each compound was measured by mass spectrometry. Selected mass ions were quantitated via computer software analysis. Using only the ion peaks associated with the naturally enriched volatile products insured that enrichment levels were not over estimated.

Results: Highly enriched $^{13}CO_2$ at atmospheric concentrations was added to the chambers containing the top undamaged leaves of both the damaged and the control plant during the same time that volatiles were collected. As indicated in Fig. 2, a high level of ^{13}C was rapidly incorporated into all the systemically released volatiles. These results clearly indicate that the volatiles released systemically in response to herbivore damage are synthesized *de novo* in the undamaged leaves from which they are released and distal from the site of damage. For (*Z*)-3-hexenyl acetate the M+2 and M+3 ions corresponding to one and two ^{13}C atoms added to the molecule are the predominantly labeled molecular ions. This suggests that there is site specific incorporation of the ^{13}C label with the acetate portion becoming heavily labeled while the hexenyl portion remains unlabeled. In fact the m/z 83 through 83 + n associated with the hexenyl fragment is the same pattern in the labeled and unlabeled molecule indicating no detectable enrichment of the hexenyl portion of the molecule. These results indicate that the lipoxygenase pathway is activated in the undamaged leaves distal from the site of feeding damage, but that the linolenic acid is obtained from storage rather than being synthesized *de novo*.

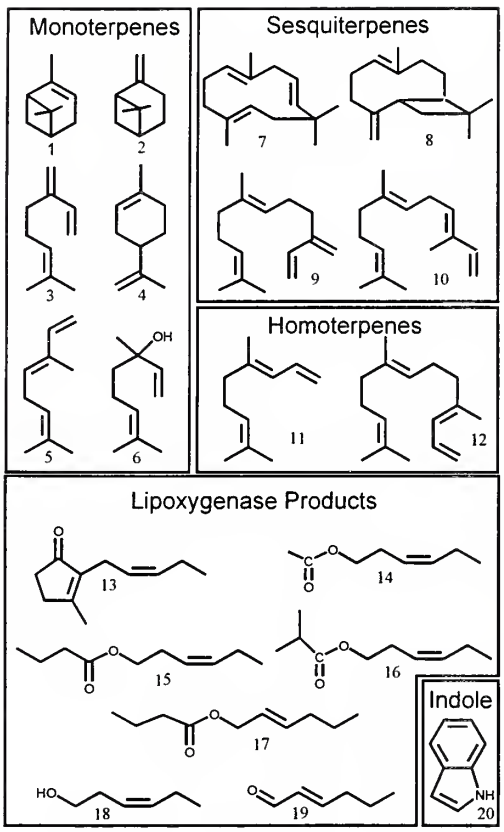

Figure 1: Compounds detected in head space volatiles collected from cotton plants damaged by beet armyworm larvae feeding on leaves. Systemic volatiles are biosynthesized *de novo* just prior to release and include: *(E)*-β-ocimene **5**, linalool **6**, *(E,E)*-α-farnesene **9**, *(E)*-β-farnesene **10**, *(E)*-4,8-dimethyl-1,3,7-nonatriene **11**, *(E,E)*-4,8,12-trimethyl-1,3,7,11- tridecatetraene **12**, and *(Z)*-3-hexenyl acetate **14**.

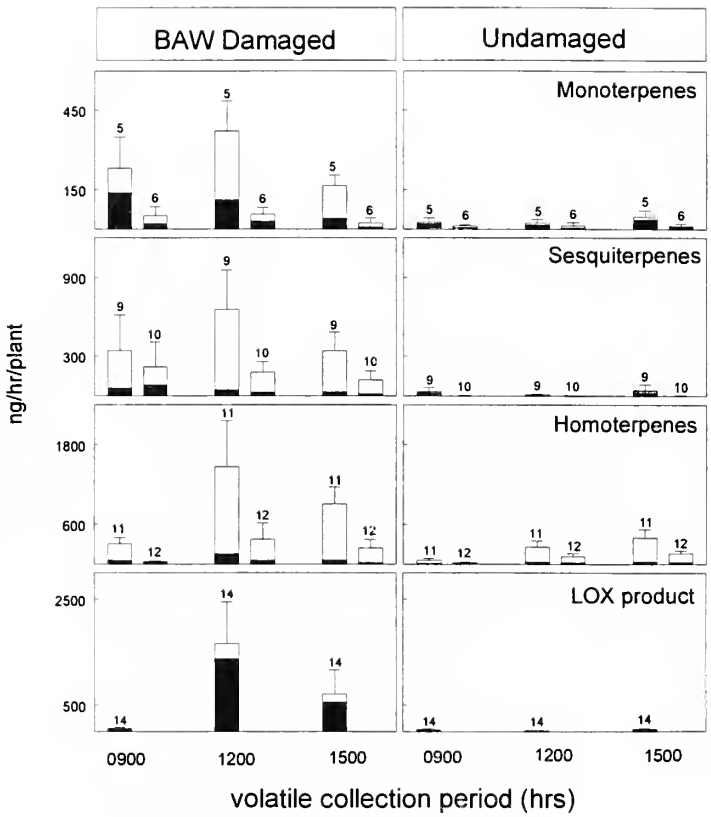

Figure 2: Volatiles released systemically from cotton plants with (BAW damaged) and without (undamaged) beet armyworm larvae feeding on the lower leaves. Uncolored portion of bars represents ^{13}C-labeled product and black areas represents non-labeled product. See figure 1 for numbering of compounds; mean (± s.e.m.) volatile release is shown with each data point, (n = 3).

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DETERMINATION OF THE PRESENCE OF PHEROMONOTROPIC NEUROPEPTIDES IN THE CEPHALIC GANGLIA OF *ANASTREPHA SUSPENSA*

P.E.A. Teal

Objectives: To determine the endogenous neural and hormonal mechanisms which regulate sex pheromone biosynthesis in *Anastrepha suspensa*

Methods: In studies to isolate and identify pheromonotropic compounds from the brain of males of *A. suspensa* the tissue was excised and extracted in acidic buffer. The extracts were concentrated to dryness and suspended in physiological saline for bioassays. Bioassays for pheromonotropic activity were conducted by injecting females of *Heliothis virescens* during the day when no pheromone is normally present in the gland, with extracts of brains of fruit flies. The sex pheromone glands of the moths were excised and extracted 1 hour after injecting the brain extracts. Pheromone gland extracts were subjected to chromatographic analysis to determine the amount of sex pheromone present. The biologically active material was also subjected to ELISA studies to determine if the active compounds had sequence homology with pheromone biosynthesis activating neuropeptide (PBAN).

Results: Extracts of the cephalic ganglia of males of *A. suspensa* stimulated females of *H. virescens* to produce sex pheromone during the photophase, when no pheromone is normally present in the pheromone gland. Dose response studies indicated that the amount of pheromone increased in a linear fashion with increasing amounts of fruit fly brain extracts up to one brain equivalent. The amount of pheromone present in extracts from females injected with one brain equivalent was the same as that present in extracts obtained from moths injected with five pmol of synthetic PBAN. The amounts of pheromone present in extracts obtained from females injected with five and 10 brain equivalents was lower than that obtained from insects injected with one equivalent. ELISA studies comparing fruit fly brain extracts with HEZ-PBAN indicated that the brain extracts contained material having some sequence homology with the synthetic PBAN fragment. About 0.6 pmol of immunoreactive peptide was present in an individual brain extract.

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DEVELOPMENT OF A TECHNIQUE FOR DIRECT IDENTIFICATION AND QUANTITATIVE ANALYSIS OF INSECT JUVENILE HORMONES BY CHEMICAL IONIZATION MASS SPECTROSCOPY

P.E.A. Teal

Objectives: To develop a technique that allows for direct quantitative analysis of juvenile hormones using chemical ionization mass spectroscopy that does not rely on formation of derivatives.

Methods: GC-MS analysis was carried out using a Finnigan-Matt ITS 40® ion trap MS operated in the chemical ionization mode (CI) and interfaced to a Varian Star 3400 GC. Conditions of chromatography were: initial injector temperature = 40° for 20 sec; injector temperature increased at 170°/ min to 270°; initial column temperature = 40° for 5 min; column temperature increased at 5°/ min to 210°; He carrier gas linear flow velocity = 24 cm/sec; GC-MS transfer line temperature = 230°. Under these conditions farnesyl acetate eluted at 32.3, JH III at 33.5, JH II at 35.4 and JH I at 37.3 min respectively. MS operating conditions were: multiplier voltage = 1900 volts; manifold temperature = 130°; emission current = 16 µ amps; mass acquisition range = 60-350 amu; scan rate = 1 sec; scan mode = chemical ionization; isobutane reagent gas.

A mixture containing 200 pg of each JH and the internal standard (farnesyl acetate) determined cleavages that resulted in production of specific ions, and selected six diagnostic ions for each compound for use in quantitative analyses. Then a series of analyses in which the amounts of the JH homologs were decreased were conducted. The minimum amount of each JH homolog analyzed was 0.01 pmol. Intensities of each diagnostic ion, determined after subtraction of background ions, were plotted against concentration and regression equations were calculated. Relative abundances of the diagnostic ions, as percentages of the most intense ion (base peak), were calculated for each compound at each concentration and were compared to the mean relative abundances calculated for all concentrations using a T-test (p=0.05).

Results: Analysis over a mass range of 60-350 amu allowed for identification of as little as 0.01 pmol of JH I, JH II and JH III. Quantitative analysis was based on the relative abundances of six diagnostic ions for each homolog. The ratio of diagnostic ions did not vary significantly over a range of concentrations from 2.66-200 pg, but the intensities of these ions increased in a linear fashion with increasing amounts of analyte. No discrimination due to disparate ratios of the individual JH homologs was found when analyzing samples differing in concentration by at least five fold. The use of this technique allows for facile, concrete identification and quantitation of biologically relevant amounts of JH and is at least as sensitive as other methods currently in use. Additionally, the ability to analyze samples without derivitization or fractionation by chromatographic methods, coupled with data acquisition over a broad mass range provides levels of accuracy and confidence greater than those of other methods.

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ISOLATION AND IDENTIFICATION OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDES FROM *MAMESTRA BRASSICA*

P.E.A. Teal, and A. Fonagy[1]

Objectives: To isolate and identify pheromonotropic neuropeptides from the cephalic ganglia of *Mamestra brassica*.

Methods: The brains were dissected from the heads of adult females and homogenized and extracted in aqueous 0.1M acetic acid. The extracts were bioassayed for pheromonotropic activity by injection into females and by ELISA analyses using an antibody binding to the C-terminal pentapeptide fragment of PBAN. The extracts were subjected to solid phase extraction using ion exchange and reversed phase systems. Active fractions from solid phase extraction were subjected to semipreparative HPLC separation using a reversed phase column. Active areas from the semipreparative HPLC separations were subjected to analytical HPLC separations using reversed phase and inversed gradient reversed phase protocols with fractions from each separation being assessed for bioactivity. Final purification was accomplished via microbore HPLC using a reversed phase column. Sequence analysis was accomplished using standard Edman degradation protocols and mass spectra were obtained using a MALDI spectrometer.

Results: To date one peptide has been purified to homogeneity and subjected to structural analyses. Mass spectral analysis of this peptide indicated a mass of 2137.75. Sequence analysis yielded the following partial sequence: SLAYVQKVF-... Additionally the C-terminal region reacted with the antibody indicating the following sequence: ...-FXPRLamide. Based on these data and homologies with other pheromonotropic neuropeptides the following sequence was deduced: SLAYVQKVFENVEFVPRLamide. The synthetic peptide had mass spectral characteristics identical with the natural product and was pheromonotropic.

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INDUCTION OF PHEROMONE PRODUCTION IN FEMALES OF *HELIOTHIS VIRESCENS* BY TOPICAL APPLICATION OF AN AMPHIPHYLIC PSEUDOTETRAPEPTIDE ANALOGS OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE

P.E.A. Teal and R.J. Nachman[1]

Objectives: To design and develop synthetic analogues of insect neuropeptides that penetrate the insect cuticle and maintain bioactivity.

Methods: Pseudotetrapeptide analogs of the C-terminal active core (FSPRLamide) of pheromone biosynthesis activating neuropeptide (PBAN) were synthesized by replacing phenylalanine with either hydrocinnamic acid, 9-fluoreneacetic acid or 1-pyrenebuteric acid. Pheromonotropic activity of the analog was assessed in injection bioassays in which females of *Heliothis virescens* were injected with different doses of the analog or PBAN. Females were incubated for 1h after injection and then the sex pheromone glands were excised and extracted in hexane containing internal standards. The extracts were then analyzed by capillary gas chromatography to determine the amount of pheromone present. Topical application studies were conducted by applying various doses of the pseudotetrapeptide analog or PBAN to the descaled abdomen in water for periods of up to 24h . After incubation the sex pheromone glands were excised, extracted and the extracts analyzed for the amount of pheromone present as above.

Results: The pseudopeptide analogs were found to be 10 fold more potent than PBAN in stimulating pheromone production when injected into females of *H. virescens*. Thus, 0.5 pmol of the pseudotetrapeptide analog stimulated production of as much pheromone as did 5.0 pmol of synthetic PBAN. Additionally, they stimulated pheromone production when applied topically in water to the abdomen. The amount of pheromone present in extracts increased in a linear fashion when doses of between 0.01 - 100pmol were applied and remained constant at doses from 100 - 2000 pmol. PBAN did not stimulate production of pheromone when applied topically at any dose. Temporal response studies indicated that pheromone production was maintained for periods of up to 20 h after topical application of the analogs.

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IDENTIFICATION OF VOLATILE CHEMICALS FROM *ENTEROBACTER AGGLOMERANS* (ENTEROBACTERIACEAE) THAT ARE ATTRACTIVE TO *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE)

N.D. Epsky, R.R. Heath, B.D. Dueben, C.R. Lauzon,[1] A.T. Proveaux and G.B. MacCollom[2]

Objective: Identify, quantify and formulate attractant chemicals released from *Enterobacter agglomerans*, a bacterium that has been isolated internally from adults as well as fruit infested with larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) and other pest fruit flies.

Methods: Headspace volatiles were collected from *E. agglomerans* grown for 24 h on tryptic soy agar plates and were analyzed by capillary gas chromatography - mass spectroscopy. Laboratory flight tunnel bioassays were conducted to verify the attractiveness of the identified chemicals for female *A. suspensa*.

Results: 3-methyl-1-butanol and ammonia were identified as the two primary volatile chemicals released from active cultures of *E. agglomerans*. Chemical analysis indicated that no 3-methyl-1-butanol and very little ammonia was released from sterile tryptic soy agar plates. *E. agglomerans* - inoculated tryptic soy agar plates, however, released an average of 1.5 µg/h 3-methyl-1-butanol and 332.9 µg/h ammonia. 3-methyl-1-butanol lures were formulated using a membrane-based system to provide a constant release rate of synthetic chemical. Release rates from the lures ranged from 1.23 ± 0.30 ng/h to 12.16 ± 2.76 µg/h. Ammonia is a known attractant for a number of fruit fly species. However, in laboratory trials, the combination of 3-methyl-1-butanol and ammonia was more attractive than ammonia alone. Development of the 3-methyl-1-butanol lures will allow testing of these microbial attractants for a variety of pest fruit flies.

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ADDING METHYL-SUBSTITUTED AMMONIA DERIVATIVES TO A FOOD-BASED SYNTHETIC ATTRACTANT ON CAPTURE OF THE MEDITERRANEAN AND MEXICAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

R.R. Heath, N.D. Epsky, B.D. Dueben, J.Rizzo[1] and F. Jeronimo[1]

Objective: Field trials were conducted in Guatemala to determine the effect of addition of methyl-substituted ammonia derivatives (methylamine, dimethylamine or trimethylamine) to a food-based synthetic attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Mexican fruit fly, *Anastrepha ludens* (Loew).

Methods: Research was conducted to determine the effects of the addition of methylamine, dimethylamine, and trimethylamine on the capture of wild *C. capitata* and *A. ludens* in traps baited with ammonium acetate and putrescine. Field trials were conducted in a mixed planting of coffee and citrus in Finca Silmar located near Palin, Guatemala. Three tests were conducted. The first was a comparison among methyl-substituted ammonia in combination with ammonium acetate and putrescine lures. The second was a test of trimethylamine synergy, and the third was a comparison among trap types.

Results: Addition of trimethylamine to traps baited with ammonium acetate and putrescine increased capture of *C. capitata*, but not *A. ludens*, over traps baited with ammonium acetate and putrescine alone, in all tests. Addition of methylamine or dimethylamine had no effect on either species. More *C. capitata* were captured in adhesive paper cylindrical traps baited with tested combinations of synthetic lures than in McPhail traps baited with liquid protein bait (torula yeast solution). In tests conducted during the dry season in Guatemala, more female *A. ludens* were captured in liquid protein-baited McPhail traps than in adhesive paper cylindrical traps baited with ammonium acetate, putrescine, and trimethylamine. However, during the rainy season, adhesive paper cylindrical traps baited with ammonium acetate, putrescine, and trimethylamine captured significantly more female *A. ludens* than liquid protein-baited McPhail traps. No *C. capitata* were captured in traps baited with trimethylamine alone, indicating that trimethylamine is a potent synergist to ammonium acetate and putrescine for the capture of *C. capitata*.

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OPTIMIZATION OF TRAPS BAITED WITH FOOD-BASED SYNTHETIC ATTRACTANT TO REPLACE LIQUID PROTEIN-BAITED MCPHAIL TRAPS FOR DETECTING, MONITORING AND SUPPRESSING POPULATIONS OF THE CARIBBEAN FRUIT FLY

R.R. Heath, N.D. Epsky, R.M. Baranowski[1], M.K. Hennessey[2] and T. Holler[3]

Objective: To evaluate a food based synthetic attractant in comparison to standard liquid protein-baited McPhail traps for the capture of the Caribbean fruit fly in different fruit tree hosts.

Methods: Field trials were conducted in South Florida. A synthetic attractant, which is composed of ammonium acetate and putrescine, is based on chemicals that are released from conventional liquid protein baits. Preliminary tests indicated that this synthetic attractant, when used in either glass McPhail traps or in plastic cylindrical McPhail traps (International Pheromones McPhail trap) with water in the base, was competitive with standard liquid protein-baited traps. Initial tests were conducted in guava. Research was expanded to include tests in additional fruit tree species (loquat and Surinam cherry, commercial grapefruit).

Results: The plastic cylindrical McPhail trap baited with the food-based synthetic attractant captured about 70% of the caribflies in tests in loquat. Trap capture with the synthetic lures was equal to or greater than trap captures with protein solutions in tests conducted in Surinam cherry, grapefruit, and guava. The plastic cylindrical trap used with the synthetic attractants also proved to be more user friendly than the other trap/lure combinations tested. The synthetic lures proved to be effective in field trials for six weeks, while it was necessary to replace the protein baits on a weekly basis. Furthermore, traps baited with the synthetic lure also captured significantly fewer non-target insects than did the protein baits, making the traps much easier for field personnel to service.

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FIELD EVALUATION OF FEMALE-TARGETED TRAPPING SYSTEMS FOR *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN SEVEN COUNTRIES

N.D. Epsky, J. Hendrichs,[1] B.I. Katsoyannos,[2] L.A. Vásquez,[3] J.P. Ros,[4] A. Zümreoğlu,[5] R. Pereira,[6] A. Bakri,[7] S.I. Seewooruthun[8] and R.R. Heath

Objective: Field trials were conducted in Greece, Honduras, Mauritius, Morocco, Portugal, Spain and Turkey to compare captures of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), among several types of traps baited with female-targeted lures.

Methods: Field studies were conducted in seven countries under an FAO/IAEA Coordinated Research Program to develop female-targeted *C. capitata* trapping systems for practical use with SIT control or eradication programs. Field trials were conducted in *Citrus* orchards, although tests were also conducted in other *C. capitata* hosts. Female-targeted trapping systems included: food-based synthetic attractants of ammonium acetate and putrescine alone (two component lure) and in combination with trimethylamine (three component lure) tested in either wet traps (with water) or dry traps (with pesticide or sticky insert); Fruitect traps baited with proprietary liquid protein bait; and McPhail-type traps baited with an aqueous solution of NuLure and borax, which is the standard female-targeted trapping system for *C. capitata*.

Results: More females, as indicated by relative trap efficiency, were captured in traps baited with the three component lure than in other female-targeted traps in three countries, and they captured more females or equal percentages of females as the standard NuLure/borax-baited traps in five of the countries. Females accounted for 48 - 90% of the total capture in the female-targeted trapping systems. Traps baited with the three component synthetic lure were more *C. capitata* specific than the other female-targeted trapping systems. *C. capitata* population levels, as indicated by average (SD) number of males captured in male-targeted trimedlure-baited Jackson traps, varied from 0.2 (0.10) - 54.4 (17.49) flies per trap per day.

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ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *Schistocerca americana* ORAL SECRETIONS

H.T. Alborn, T. Hansen and J.H. Tumlinson

Objective: To isolate and identify the substance(s) in the oral secretions of *Schistocerca americana* that induces plants to biosynthesize and release volatile compounds.

Methods: *Schistocerca americana* nymphs and adults obtained from Dr. John Capinera, Dept. of Entomology and Nematology, University of Florida, were maintained under a 14:10 LD cycle at 60 % RH and 25 °C and fed corn seedlings. Oral secretion was collected from the grasshoppers by gently squeezing them and drawing the oral secretion into a capillary pipette under a slight vacuum. Oral secretion is stored at -70°C. Crude oral secretion is centrifuged at 16,000*g* for 30 min to remove solids and the supernatant is then filtered through a 0.22 µm sterilizing membrane. . Separation and purification of the active compound(s) is achieved by solid phase extraction on a C_{18} column, followed by a series of separations on C_{18} reverse phase HPLC columns with different mobile phases. A bioassay which consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings is used to monitor fractionation of the oral secretion. An amount of each fraction equal to 10 µl of crude oral secretion is added to 500 µl of 50 mM pH 8 phosphate buffer. A 9- to 10-day-old corn seedling is cut off above the root with a razor blade and the cut end immersed in the buffer solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over a period of 12 hr in complete darkness. Then the seedling for each treatment is placed in a glass volatile collection apparatus (15 cm long, 3 cm id) under artificial light. Purified, humidified air is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. Then the adsorbent is extracted with 150 µl of methylene chloride and the extract analyzed by capillary GC.

Results: The crude oral secretion of *S. americana* induces corn seedlings to produce and release the same volatile compounds in similar proportions, but in approximately 6-fold larger quantities, than the oral secretion of *Spodoptera exigua* caterpillars. The active compound(s) are eluted from the solid phase extraction column with a 1:1 water/acetonitrile solution. In contrast to volicitin, the active compounds cannot be extracted into methylene chloride from an acidified aqueous solution. This, and different retention characteristics on reverse phase HPLC columns indicate a structure different from volicitin.

IMPORTED FIRE ANT

AND

HOUSEHOLD INSECTS

CRIS - 6615-32000-026-00D--Integrated Control of Insect Pests in an Urban Environment with Emphasis on Roaches, Fleas, and Ants

CRIS - 6615-32000-028-00D--Fire Ant Ecology and Management

CRIS - 6615-32000-029-00D--Biological Control of Fire Ants

CRIS: 6615-32000-026-00D

IMMUNOLOGICAL ANTIGEN DETECTION SYSTEM FOR SPATIAL ASSESSMENT OF POTENTIAL COCKROACH ALLERGENS IN STRUCTURES

R.J. Brenner, M.C. Anderson[1] and R.M. Helm[2]

Objectives: The objectives were to develop a polyclonal, rabbit-anti-cockroach ELISA-inhibition detection system for the "soup" of cockroach proteins that may elicit allergic disease, and to use spatial analysis to demonstrate that this detection system allows a comparative assessment of mitigation efficacy in a structure with a known past history of German cockroach infestation.

Methods: Rabbit-anti-cockroach IgG (antiserum) was provided by FDA's Laboratory of Standards and Testing. IgG antibodies were raised to a "standardized cockroach debris" prepared from debris remaining from rearings of German cockroaches, *Blattella germanica*, in Gainesville. To determine cockroach-hour equivalents, 50 mixed stages of German cockroaches were held without food and water for 3-96 hours. Cockroaches were removed, and a Q-tip moistened with phosphate buffered saline (PBS) was used to swab internal surfaces of the petri dish. Swabs were extracted at FDA overnight in 1 ml of 50% PBS/glycerine. Proteins extracted from these swabs served to compete with standardized cockroach debris coating the microtiter plate cells in the ELISA-inhibition assay. A plate reader was used to record the optical density, which was then converted to % inhibition. This was converted to cockroach-hr equivalents, based on the timed samples. Environmental samples were taken from an ARS experimental structure (8 x 13 m) that had a past German cockroach history of ca. 2500 mixed stages for 5 months; no cockroaches had been in the structure for 5 years. Each sample consisted of a swab from a 10 x 10 cm area of the floor, countertop, table top, or appliance top. For each spatial analysis 110 samples were taken; spatial analysis was used to generate maps showing distribution of cockroach antigens before and after mitigation interventions consisting of vacuuming and cleaning with a no-rinse cleaner, and finally after cleaning with a full-rinse cleaner.

Results: The antigen detection system is highly sensitive to cockroach antigen levels, at least as low as 150 cockroach-hr equivalents, even after cockroaches had been absent from the building for at least 5 years (Fig. 1). A rigorous vacuuming and cleaning with a no-rinse cleaner reduced antigen levels enormously, and after a second cleaning with a full-rinse cleaner, all 110 environmental samples were at or below "zero cockroach-hr equivalents". This study reveals (1) the profound persistence of potential cockroach allergens indoors, (2) therefore, the need to determine the entire spatial distribution of these potential allergens for mitigation, and (3) the combination of spatial analysis for pre- and post-intervention antigen levels now allows further research to be conducted to develop technologies and strategies for indoor cockroach allergen management.

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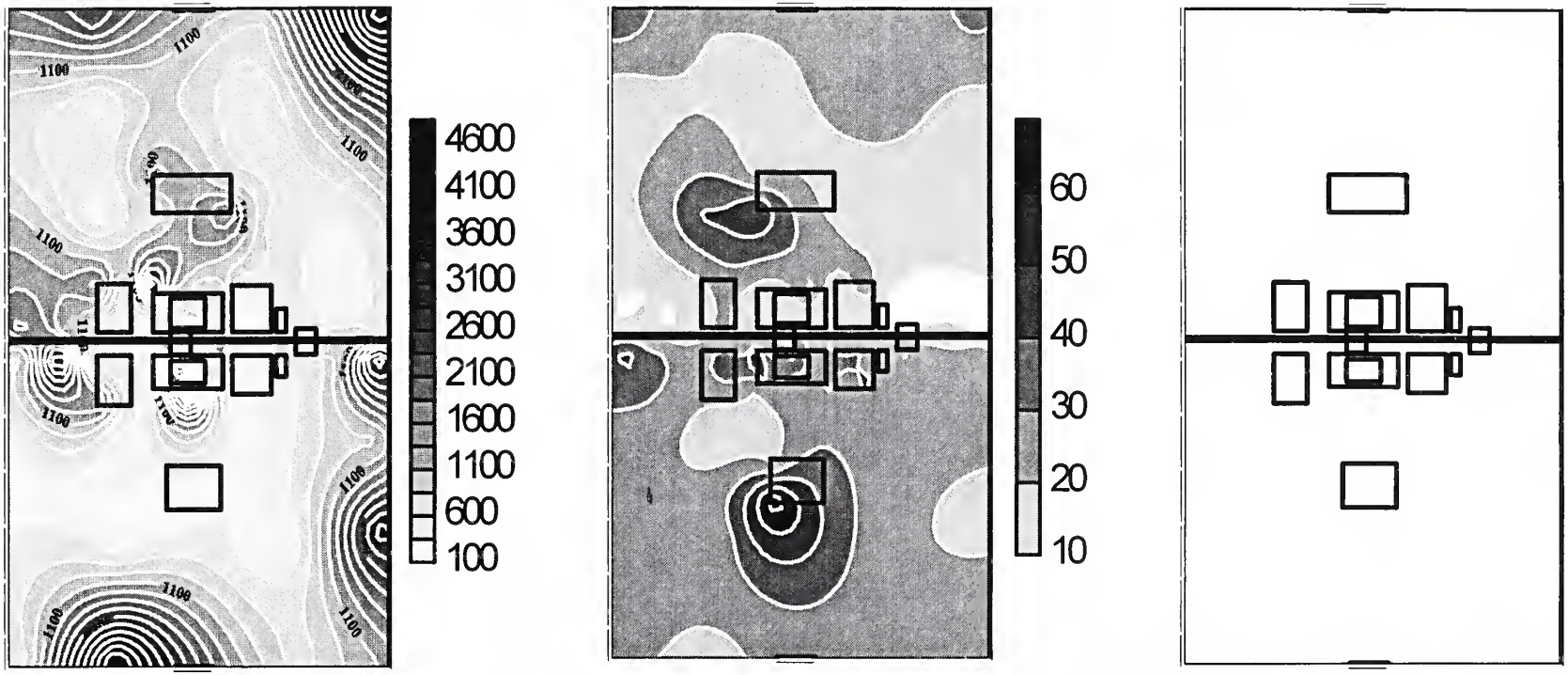

Fig. 1. Spatial distribution of German cockroach antigens (potential allergens) based on 110 swab samples of 10 x 10 cm areas within 12 x 8 m experimental structure in Gainesville. Black boxes show relative location of countertops, stove, refrigerator, sink, wall cabinets, trash can, and table. Extracts from swabs were analyzed using an ELISA-inhibition assay. Pretreatment sampling (left) revealed persistence of high antigen levels even though all cockroaches had been removed at least 5 years prior to sample. Middle panel shows effect of vacuuming and using a non-rinse cleaner; some antigens still remained, but levels were markedly reduced. Right panel shows results of 110 samples following use of a full-rinse cleaner in the same structure; all samples were at or below the "zero cockroach-hr equivalent" (bar scale). All assays were conducted as blind tests at FDA's Laboratory of Standards & Testing, Rockville, MD.

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SITE-SPECIFIC ESTIMATES OF TRANSISSION THRESHOLDS FOR DENGUE[1]

D.A. Focks and R.J. Brenner

Objectives: Earlier this year in Trinidad, we reported the development of an entomological survey method involving absolute counts of *Aedes aegypti* pupae useful for assessing the risk of dengue transmission. The present report documents, for the first time for any vector-borne disease, the development of transmission thresholds. The estimates are based on the pupal survey in Trinidad, herd immunity, and ambient air temperatures. In an analysis of a virgin soil epidemic of dengue in Honduras, the transmission threshold was < 0.25 *Ae. aegypti* pupae per person (Focks et al. 1995). In our Trinidad survey, we noted that every site exceeded this value by a considerable margin. In light of the known influence of temperature and seroprevalence of antibody, how should such levels in Trinidad be interpreted? Are all sites susceptible to epidemics should dengue virus be introduced? How acute would epidemics be?

Methods: To answer such questions, we used the dengue models developed at CMAVE to estimate a transmission threshold for Trinidad. We began with a definition of an epidemic that is arbitrary but useful from a public health point of view- we considered any single year where seroprevalence rises by at least 10% to be an epidemic year. Just how many mosquitoes per person are required to support this level of transmission is a function of many factors but the ones considered key determinants are number of introductions during the year, seroprevalence of dengue antibody, and ambient air temperatures. Using the dengue transmission model (DENSiM), we made iterative runs using various constant ratios of *Ae. aegypti* females and humans under conditions of monthly introductions of a single viremic individual and an initial seroprevalence of dengue antibody to be 0, 33, or 67%. We used the entomological model (CIMSiM) to estimate several entomological parameters that are used by DENSiM- the rates of gonotrophic development and adult survival, and the average adult female weight using temperatures based on weather data from the capital, Port of Spain.

Results: Based on this analysis, we estimate the transmission thresholds in Trinidad necessary to produce a 10% annual rise in seroprevalence to be 0.10, 0.14, and 0.32 pupae per person for initial rates of seroprevalence of 0, 33, and 67%, respectively. Based on these thresholds, virtually all sites are significantly at risk for dengue transmission should virus be introduced. This is true even in the environs of the capital where sanitation results in the lowest levels of *Ae. aegypti* in the country- here entomological counts are still, depending on initial seroprevalence, some 8- to 23-fold higher than necessary to support endemic transmission. The thresholds are substantially higher if by threshold we mean the number of pupae per person necessary to lead to epidemic at least 50% of the time due to a *single* introduction during the year. Again, using initial seroprevalences of 0, 33, and 67%, the number required was 0.19, 0.21, and 0.42 pupae per person. Software being developed under SERDP will enable this type of analysis to be made easily for any location where pupal surveys have been conducted.

1Research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic environmental Research and Development Program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

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SPATIAL ANALYSIS APPLIED TO THE CONTROL AND RISK ASSESSMENT OF DENGUE IN TRINIDAD

D.A. Focks and R.J. Brenner

Objectives: In recent work we 1) developed an epidemiologically significant entomological and demographic survey method for dengue risk assessment, and 2) using models developed at CMAVE, integrated the entomological/demographic survey data with serologic and air temperature data to provide estimates of dengue transmission thresholds for a specific location. This report discusses using this earlier work to provide dengue mitigation recommendations on a spatial basis. This work represents the final phase of a major thrust of our SERDP efforts, to provide spatially-based risk assessment and mitigation analyses and recommendations for a deployment disease of interest to DoD. Similar work needs to be focused on other DoD-relevant illnesses, e.g., malaria and Lyme disease.

Methods: Again, for demonstration purposes, we use the island-wide survey data for Trinidad. This survey indicated that not only did different sites have significantly different levels of *Aedes aegypti*, but that the types and frequencies of containers responsible for their production varied remarkably from site to site. In a targeted source reduction program for Trinidad where container types were selected on the basis of vector productivity, the classes of containers represented by *Small misc., Buckets, Tubs, etc., Tanks, and Drums* would be key receptacles to target. We do not consider here a strategy of insecticide sprays against adults because they have been shown to be ineffective, prohibitively expensive, and otherwise unsuitable for long-term control for DoD deployments. Below we present an analysis of the efficacy of 2 strategies of source reduction that are similar to ongoing efforts in various parts of the world for dengue control.

Results: In our first example, *Strategy 1*, we evaluate a general, sanitation program designed to eliminate abandoned tires (*Tires)* and the most important single type of container in Trinidad, discarded trash (*Small misc.*). This could represent a government-funded effort aimed at containers not used domestically to store water. Because sites differ in the types and numbers of containers present, a sanitation program would be expected to have spatially variable results. For example, tires and trash were virtually non-existent in one town whereas in another town these types accounted for >60% of all *Ae. aegypti* production. The reductions in pupae per person in the first town remained unchanged under *Strategy 1* but the second town declined from about 140 to 54 pupae per person. What would these reductions do to the probability of transmission? Figure 1 presents spatially the ratio of the observed pupae per person and the transmission threshold of 0.2 pupae per person. The sanitation program, even if completely effective, would not result in prophylactic levels of dengue vectors anywhere in Trinidad. Well, how about a more vigorous approach? Let's assume the existence of an adequate potable water supply system thought the island, adequate such that all domestic water storage containers (*Indoor and Outdoor Drums, Buckets,* and *Tires*) could be eliminated. Combine this with the on-going sanitation program and further reductions could be expected (*Strategy 2*). Note again, the projected levels of pupae per person are still high enough to support transmission in all but 2, perhaps 4, of the 16 sites.

[1]Research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic Environmental Research and Development Program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

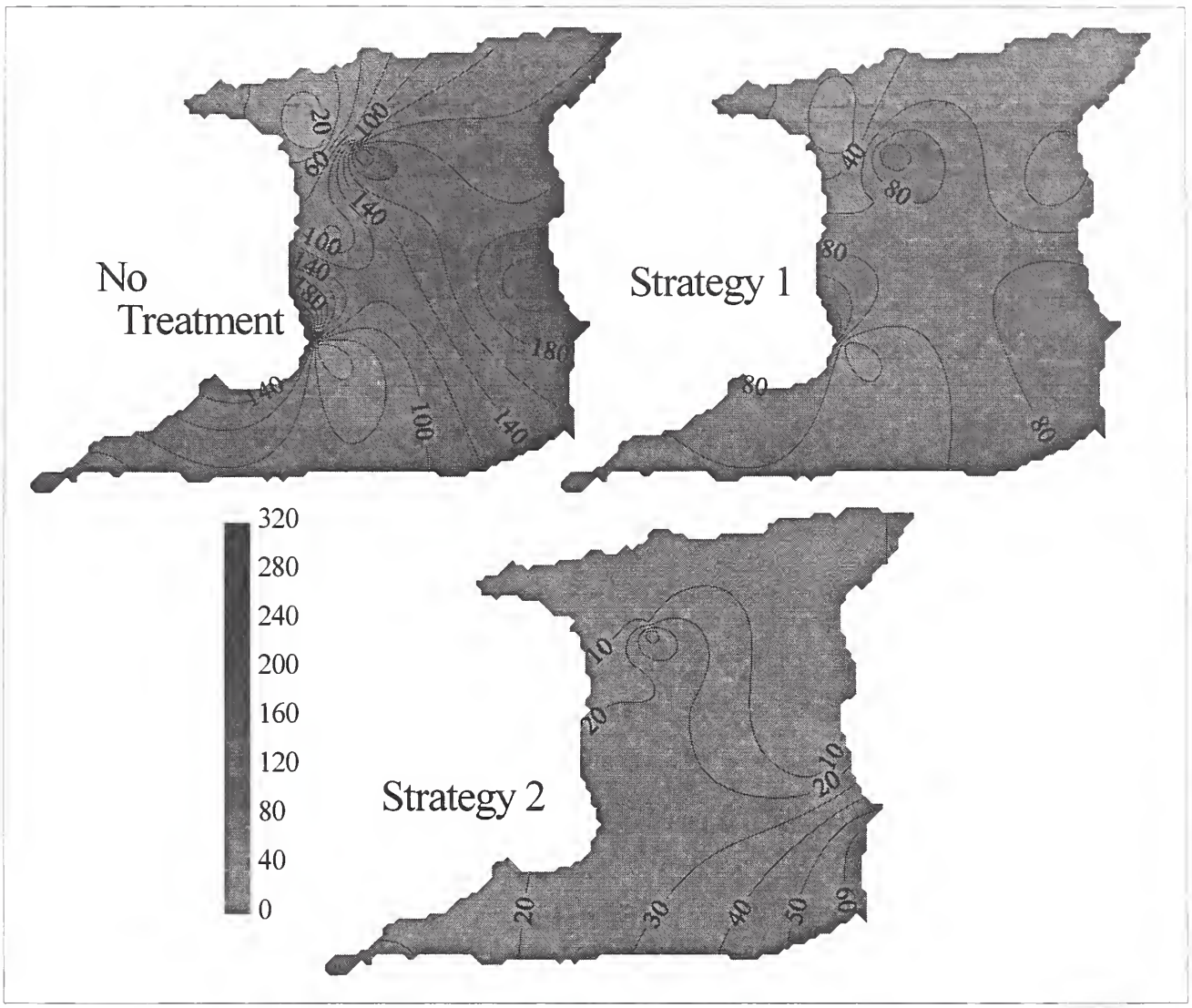

Figure 1. Plots of the spatial distributions of the ratios of the observed pupae per person and a transmission threshold of 0.2 pupae per person, the value estimated to result in an epidemic on 50% of occasions when a single viremic individual is introduced and seroprevalence ranges between 0 and 33%. *Strategy 1* refers to the complete elimination of all of the containers classified as *Small miscellaneous* and *Tires*; this type of cleanup is simply basic sanitation, the removal on unnecessary water-holding containers in the environment. *Strategy 2* involves the additional control of containers that are necessary to store domestic water, containers that could be reduced if a potable water supply was in place throughout Trinidad. *Strategy 2* involves the control of the following types: *Small miscellaneous, Tires, Indoor and Outdoor Drums, Buckets, and Tanks.*

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DEVELOPMENT OF AN EPIDEMIOLOGICALLY SIGNIFICANT SURVEY METHOD FOR DENGUE RISK ASSESSMENT FOR DOD[1]

D.A. Focks, R.J. Brenner, and D.D. Chadee[2]

Objectives: Dengue viruses cause a spectrum of illness in humans ranging from inapparent infection with flu-like symptoms to a viral syndrome of febrile illness, rash, and severe headache and muscular and skeletal pain to frank, life-threatening shock and hemorrhage. Because of the widespread distribution of these viruses and their vector, *Aedes aegypti*, dengue represents a significant concern to DoD operations. Current methods to assess dengue risk provide no actual measure of transmission risk to deployed forces nor any indication of how acute or pervasive epidemics might be. The purpose of this study was to develop a practical and epidemiologically meaningful survey for dengue permitting risk assessment and prophylactic recommendations for DoD operations when coupled with dengue transmission models developed at CMAVE.

Methods: In cooperation with the Trinidad and Tobago Ministry of Health, all of the natural and artificial containers located indoors and outdoors at more than 100 houses and compounds in each of 16 towns located throughout Trinidad were inspected to determine whether they were wet or dry and for the number of *Ae. aegypti* pupae. Statistical comparisons were made between the actual number of pupae per person and the traditional *Stegomyia* measurements, the House, Container, and Breteau indices which are based only on the presence or absence of active immatures. Trinidad is endemic for 3 of the 4 dengue serotypes.

Results: The Breteau (BI), House (HI), and Container (CI) Indices for the 16 towns were all significantly correlated ($P<0.05$) from a statistical point of view. However, the actual amount of variation (R^2, the squared correlation coefficient or coefficient of determination) in one index that could be explained by reference to another index was low and ranged between 30% (CI *vs.* HI) and 47% (BI *vs.* HI). Certainly, if the indices represented significant correlates of dengue risk, they should themselves be correlated. More important in the context of using these indices as epidemiological risk surrogates was that there was a complete lack of correspondence between the absolute measure, *pupae per person*, and any of the *Stegomyia* indices- the only statistically significant one being inappropriately negative in the case of the CI. The lack of correlation could also be visualized spatially (Fig. 1). We observed that that the spatial distribution of CI did not identify the areas of high infestation on the east coast in the county of Nariva nor the hot spot on the west coast in Victoria. The HI did somewhat better, correctly identifying coastal Nariva but inappropriately highlighting the peninsula northwest of the capital, Port of Spain. The BI perhaps fared best, yet it too inappropriately identified the northwest peninsula of Trinidad. We concluded that estimates of absolute vector density such as are available from pupal surveys are obviously to be preferred over the traditional *Stegomyia* indices. In a subsequent study, these estimates were used to provide an indication on a spatial basis of just how far, from an epidemiological perspective, the existing vector densities in Trinidad are from prophylactic levels and to provide the basis for development and evaluation of control strategies.

1Research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic Environmental Research and Development Program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

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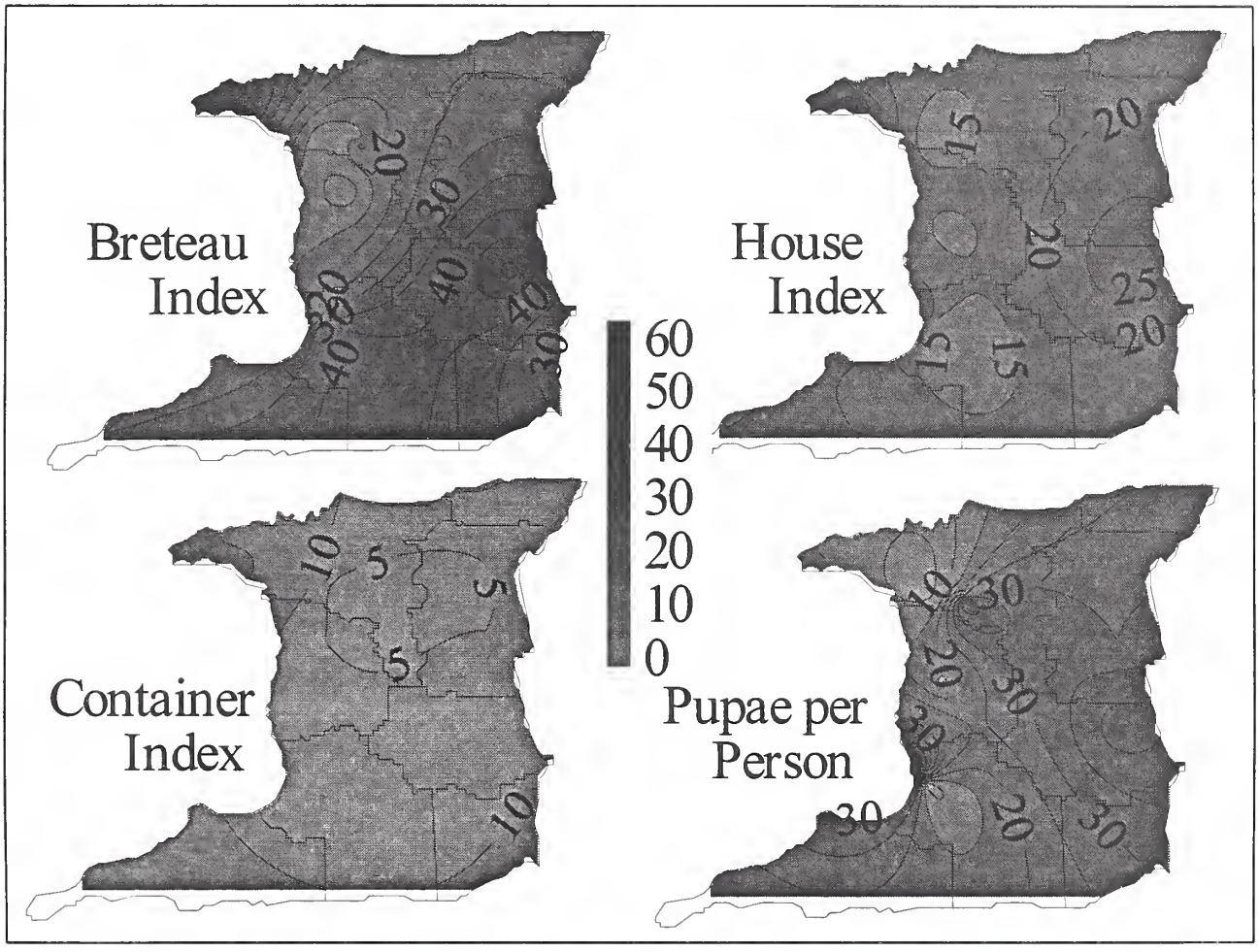

Figure 1. Comparison of the spatial distributions of the 3 *Stegomyia* indices and the absolute number of *Ae. aegypti* pupae per person.

CRIS - 6615-32000-026-00D

RISK ASSESSMENT AND MITIGATION RECOMMENDATIONS FOR SYLVAN PEST POPULATIONS OF *AEDES ALBOPICTUS* FOR THE U.S. NAVY IN HAWAII[1]

D.A. Focks, R.J. Brenner, and J.H. Trosper[2]

Objectives: The Naval Communication Area Master Station, located on the island of Oahu, is a small base sited along a narrow and cleared ridge within the Ewa Forest Preserve. The base had a history of high, year-long pestiferous levels of day-biting *Aedes albopictus* mosquitoes that were considered unacceptable. The response had been to apply aerosols of malathion weekly from truck-mounted ultra-low volume machines. A collaborative project with the U.S. Navy was designed to study the nature of the problem, to evaluate if spray operations were appropriate, and make control recommendations.

Methods: *Ovitrap Survey.* Sixty-four ovitraps were placed in shaded sites at ground level and monitored weekly for a year for *Ae. albopictus* oviposition. *Spatial analysis methods.* Spatial analyses were conducted using a commercially-available software package, *Surfer for Windows,* after the methods described by Brenner et al. (1997). We used kriging to estimate or interpolate values at unsampled points within our area of interest based upon the spatial location and value of each observation. This allowed the entire distribution of oviposition to be estimated and visualized as isolines of equal parameter density. *Simulation studies of temporal dynamics.* The average weekly oviposition of *Ae. albopictus* in the study site varied temporally. Using local weather data, we used CIMSiM, a computer simulation model developed earlier at CMAVE to partition the portion of weekly variation in oviposition that could be attributed to the sprays as opposed to being due to seasonal fluctuations in temperature and rainfall.

Results: There was a lack of substantial breeding within the base complex and the spatial distribution of oviposition argued that the biting populations of *Ae. albopictus* were the result of immigration from the surrounding Ewa Forest. CIMSiM was able to predict the temporal population dynamics of *Ae. albopictus* by reference only to seasonal changes in rainfall and temperature (Fig. 1). This result made it difficult to attribute any of the population fluctuations to the efficacy of the insecticide sprays. This conclusion was corroborated in the comparisons of the spatial and temporal distribution of oviposition as a function of treatment (Fig. 2). We concluded, therefore, that attempting control based on space sprays of insecticides was not only ineffective but inappropriate and should be discontinued. We recommended that if source reduction, the removal of forest adjacent to the base, was not a viable alternative, then, without a tangible threat of *Ae. albopictus*-borne illness, no insecticide-based control measures should be attempted on the base. In risk assessment and comparative risk reduction terms, we concluded that the minimal threat posed by the presence of *Ae. albopictus* did not justify continuing with the only available but ineffective mitigation method- an obviously cost ineffective practice that would result only in potential environmental pollution or contamination.

1 Research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic Environmental Research and Development Program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

2 James H. Trosper, then Commander, Medical Service Corps, U.S. Navy, Head, Department of Entomology, Navy Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, HI.

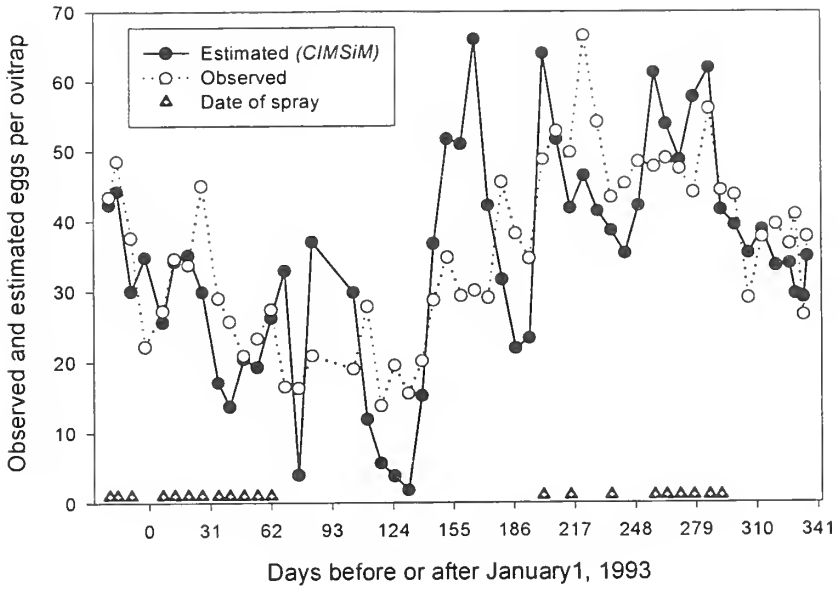

Figure 1. Plot of base-wide average observed and estimated number of *Ae. albopictus* eggs per ovitrap per week during the study period beginning November 21, 1992.

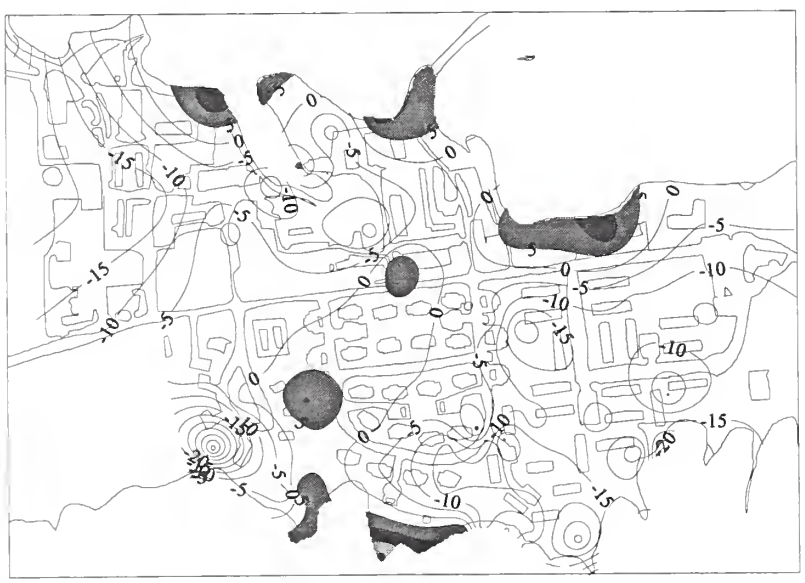

Figure 2. Ineffectiveness of treatment is shown by subtracting the average number of eggs per ovitrap during weeks when no sprays were applied from the average of weeks where treatments were made. Positive numbers indicate lower oviposition rates during weeks of sprays. Negative values appear where average oviposition was actually greater during weeks of spray operations.

CRIS: 6615-32000-026-00D

ANTAGONISM OF FIPRONIL TOXICITY BY PIPERONYL BUTOXIDE AND *S,S,S*-TRIBUTYL PHOSPHOROTRITHIOATE IN THE GERMAN COCKROACH(DICTYOPTERA: BLATTELLIDAE)

S.M. Valles, P.G. Koehler[1] and R.J. Brenner

Objective: To evaluate the toxicity of the phenylpyrazole fipronil against laboratory and field-collected strains of the German cockroach.

Methods: Dose response experiments were conducted using a standard insecticide-susceptible strain and 3 laboratory-reared, insecticide-resistant strains

Dose--mortality relationships were assessed by topical or injection method. Ten adult male cockroaches (2--3 wk old) were lightly anesthetized with CO_2, placed in petri dishes (15 by 100 mm) and subsequently treated on the 1st abdominal sternite with insecticide in 1 µl of acetone. When synergist bioassays were performed, piperonyl butoxide (100 µg per cockroach) or *S,S,S*-tributyl phosphorotrithioate (30 µg per cockroach) was applied in 1 µl of acetone to the 1st abdominal sternite 1 h before insecticide treatment.

Once dose--mortality responses had been established for the laboratory strains, the tolerance of field-collected strains of German cockroaches was evaluated. Populations were collected from homes and commercial food facilities and subsequently reared in the laboratory. After 1--4 generations in the laboratory, adult male cockroaches were topically treated with 10.2 ng (LD_{99} Orlando strain) or 16.8 ng (LD_{99} Marietta strain) of fipronil in 1 µl of acetone. Mortality was assessed 24 h after insecticide treatment.

Results: Fipronil effectively killed German cockroaches when applied in nanogram quantities. Orlando (insecticide-susceptible), HRDC (carbamate- and organophosphorus-resistant), and Village Green (pyrethroid-resistant) strains were equally susceptible to fipronil with LD_{50} values between 4.6 and 5.4 nanograms per insect. The Marietta strain (pyrethroid-, organophosphorus-, and carbamate-resistant) was 1.6 times more tolerant of fipronil compared with the Orlando susceptible strain. Five German cockroach strains collected from the field were considerably more tolerant of fipronil than Marietta cockroaches. Piperonyl butoxide and *S,S,S*-tributyl phosphorotrithioate antagonized the toxicity of fipronil in all strains evaluated. Fipronil was 2.2--3 times less toxic when cockroaches were pretreated with piperonyl butoxide or *S,S,S*-tributyl phosphorotrithioate compared with fipronil alone. These data suggest that fipronil is metabolically activated in German cockroaches, possibly via sulfone formation catalyzed by microsomal oxidases.

CRIS: 6615-32000-026-00D

TEMPERATURE EFFECTS ON Λ-CYHALOTHRIN TOXICITY IN INSECTICIDE-SUSCEPTIBLE AND -RESISTANT GERMAN COCKROACHES (DICTYOPTERA: BLATTELLIDAE)

S.M. Valles, H. Sánchez-Arroyo,[1] P.G. Koehler[1] and R.J. Brenner

Objective: Compare the effect of different temperature treatments made before and after insecticide application on λ-cyhalothrin toxicity in insecticide-susceptible and -resistant German cockroaches. We also examined the effect of the synergist, piperonyl butoxide, on λ-cyhalothrin toxicity at different temperatures in these strains.

Methods: The insecticide-susceptible Orlando and -resistant Village Green strains were used in all experiments. To assess pre-treatment temperature effects on toxicity, four groups of 100 adult male cockroaches (1-3 weeks old) were removed from a rearing tub and placed into 4 liter glass jars with cardboard harborage, #5001 laboratory rodent diet, and two cotton-stoppered 20 ml scintillation vials of water. Each jar of 100 cockroaches was held for 10 days at 19, 26, or 31°C in *Florida Reach-In* environmental chambers at 80% relative humidity on a 12:12 (L:D) photoperiod. At the end of the 10 day incubation period, the cockroaches were removed from the environmental chamber and placed into 15 by 100 mm Petri dishes (10 cockroaches per Petri dish). Cockroaches were subsequently bioassayed by topical insecticide application in 1 µl of acetone to the first abdominal sternite. Cockroaches were held at 26° C and mortality was assessed 24 hours after insecticide application.

To assess post-treatment temperature effects on insecticide toxicity, adult male German cockroaches (1-3 weeks old) were placed into Petri dishes and treated topically with insecticide as described above. At least 5 insecticide concentrations causing >0% and <100% mortality were chosen for each bioassay. Immediately after treatment, the cockroaches were placed into *Florida Reach-In* environmental chambers at 80% relative humidity on a 12:12 (L:D) photoperiod at 19, 26, or 31°C. Mortality was assessed 24 hours after insecticide application.

Results: Pre- and post-treatment temperature effects on λ-cyhalothrin toxicity were determined in an insecticide-susceptible and -resistant German cockroach strain. Acclimation at 19, 26, or 31° C for 10 days before insecticide treatment had no effect on λ-cyhalothrin toxicity in either strain. No differences were observed in aldrin epoxidase and glutathione-S-transferase activities when Orlando cockroaches were incubated for 10 days at 19, 26, and 31° C. When temperature treatment followed insecticide application, a negative temperature coefficient of toxicity (greater toxicity at lower temperature) toward λ-cyhalothrin was observed for the Orlando but not the *kdr*-type resistant Village Green cockroaches. Piperonyl butoxide synergized λ-cyhalothrin in Orlando cockroaches 3- and 5-fold at 26 and 31° C, respectively. Synergism did not occur in the Village Green strain regardless of temperature.

[1]University of Florida

CRIS: 6615-32000-028-00D

ACCEPTANCE AND FATE OF FEMALE SEXUALS IN MONOGYNE AND POLYGYNE *SOLENOPSIS INVICTA* COLONIES

R.K. Vander Meer and S.D. Porter

Objective: The imported fire ant (IFA), *Solenopsis invicta,* currently infests over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. It has also been reported in California, Arizona, New Mexico, and Maryland. The IFA is a medical, agricultural and environmental pest species. Mature IFA colonies may contain 250,000 workers and reach infestation rates of over 130 mounds per hectare. A multiple queen form (polygyne) of the IFA has been proliferating in infested areas over the last two decades. It is not uncommon to find 10s of millions of polygyne fire ants per hectare existing in one super colony with hundreds of small mounds. Control is difficult because of the large number of queens and workers that must be killed. The mechanism of polygyne colony formation and the source of queens in existing polygyne populations is of considerable interest. We measured the response of monogyne and polygyne workers to several types of female sexuals in order to better understand the possible dynamics of polygyne colony formation and the source of polygyne queens.

Methods: The aggression bioassay and olfactometer bioassay are documented in the literature. All fire ant colonies and female sexual types were obtained from the Gainesville area.

Results: Figure 1 shows the response of monogyne and polygyne workers toward intruders from the following types of female sexuals: 1) mature female alates, 2) newly mated queens introduced within three hours of mating, 3) young newly mated queens (10-14 days old), 4) old newly mated queens (post claustral colony founding stage), 5) and mature polygyne queens. Monogyne colony workers reacted aggressively to all treatments except where the intruders were female alates. This level of aggression (7-9) usually results in the death of the intruder. Polygyne workers reacted to female alates and polygyne queens at the upper level of investigative display (1-3), which would normally lead to acceptance. The other three treatment categories elicited lethal levels of aggression.

To determine if the workers' aggressive response to inseminated females is related to the "queen recognition" pheromone, we conducted olfactometer tests on all female sexual types. The olfactometer measures worker attraction, which is a behavior associated with the queen recognition pheromone. The results are shown in Figure 2. All inseminated female sexual types, except young newly mated queens, are highly attractive to workers. Alates and newly mated queens were not attractive to workers, yet young NMQs were aggressively attacked by workers, but alates were not. Thus, production of the queen recognition pheromone is not related to the increase in aggression exhibited by workers toward female sexuals after insemination.

It is unlikely that monogyne colonies are being converted to polygyne colonies through the direct adoption of any of the inseminated queens types used in our experiments. It is also unlikely that polygyne populations are replacing queens directly through adoption of any of the inseminated queens types used in our experiments.

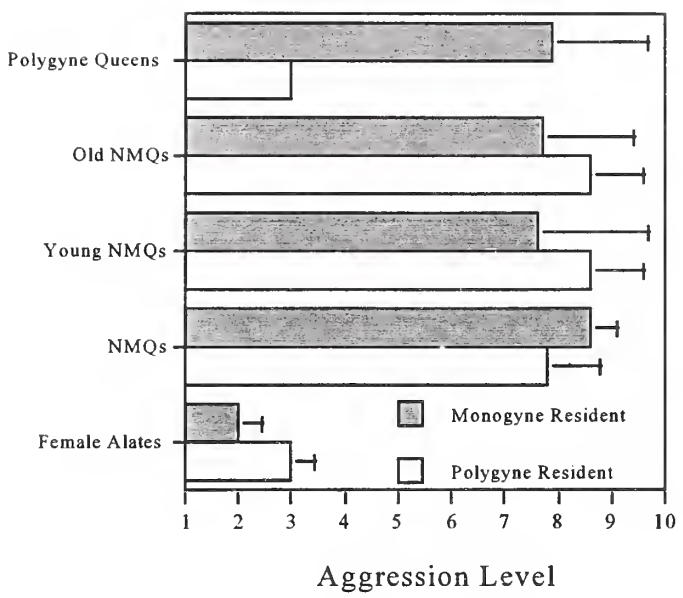

Figure 1. Aggressive response of monogyne and polygyne workers toward the following intruders types: mature female alates (uninseminated sexuals); NMQs = newly mated queens introduced within three hours of mating, young NMQs = newly mated queens (10-14 days old), old NMQs = newly mated queens (post claustral colony founding stage), and polygyne queens = mature polygyne queens. Aggression levels 1-3 = investigative; 4-6 = challenge; 7-9 = lethal attack.

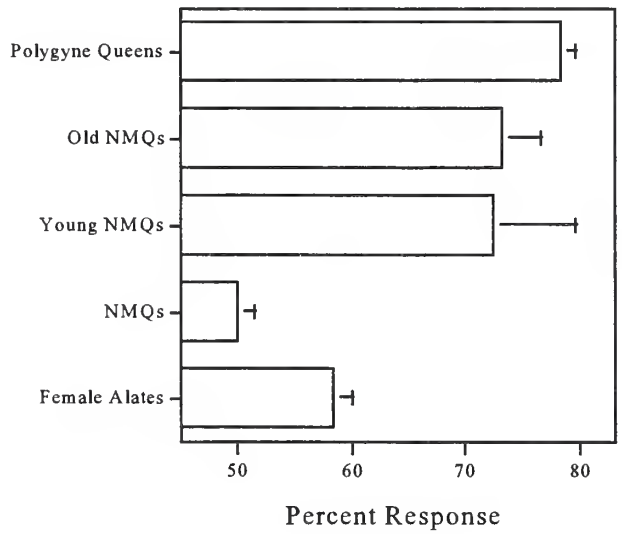

Figure 2. The female sexual types from which poison sacs were dissected and bioassayed are the same as those described in Figure 1. The bioassay is a Y-tube olfactometer that measures the ants response to volatile compounds. A response greater than 65% is significantly attractive (X^2 test).

CRIS: 6615-32000-028-00D

SEMIOCHEMICALS RELEASED BY ELECTRICALLY-SHOCKED RED IMPORTED FIRE ANTS, *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

R.K. Vander Meer, T.J. Slowik[1] and H.G. Thorvilson[2]

Objective: One of the more unexpected ways in which fire ants affect human activities is through their accumulation in and damage of electrical equipment. Large numbers of fire ants invade outdoor electrical apparatuses, creating short-circuits, fouling conductive materials, and "jamming" internal mechanisms. Earlier investigations of why this occurred demonstrated that AC frequencies, heat, ozone, magnetic fields, and wire insulation have no effect on the ants; however, the ants apparently were "attracted" to electrical fields. More recently it has been reported that the ants are not attracted to the electrical field, but instead release chemicals that excite and attract other ants to the site of release after contact with bare, bridgeable conductive surfaces, e.g. wires/contact points.

Electrical shock of worker ants causes incapacitation, death, and peculiar behaviors such as aggression towards sister workers. Ant electrification also results in both voluntary and involuntary "gaster flagging", whereby ants presumable release chemical cues at active electrical sites. Therefore, the release of semiochemicals by electrically shocked worker ants in the vicinity of infested circuitry may be an important factor in the destructive aggregations of fire ants in electrical equipment. The research presented here investigates this possibility.

Methods: Ants were electrically shocked as previously described. The alarm, olfactometer, and orientation bioassays have been described in the literature, as has the analysis of fire ant venom alkaloids and cuticular hydrocarbons.

Results: The material released by electrically shocked fire ants did not release a worker attraction response nor an orientation response, thus recruitment pheromones are not released in detectable amounts (100 pg/cm of trail).

Alarm bioassays showed that electrically shocked worker fire ants deposited material that elicited a significant alarm reaction in fire ant workers. The results are shown in Figure 1. Both the air above disturbed live fire ant workers and the air in vials in which fire ant workers were electrically shocked produced alarm reactions that were significantly greater than that of the air control ($G = 8.55$ and 7.38, respectively, $P < 0.01$). There was no difference in alarm reaction between disturbed live S. invicta workers and the headspace in the vials in which worker fire ants were electrically shocked ($G = 0.17$; $p > 0.05$; $n = 13$). There were 10 ties, two in favor of the disturbed worker ants and one replicate in favor of the treatment vials.

All 12 samples analyzed for venom alkaloids by gas chromatography had the pattern of four major piperidine alkaloids characteristic of S. invicta workers. Two of the 12 samples analyzed also had detectable amounts of the five cuticular hydrocarbons specifically associated with S. invicta. The amount of alkaloid in six samples was quantified through the addition of a heptadecane internal standard, thus the amounts reported are relative to the detector response of the hydrocarbon standard. The mean amount of alkaloid (± SE) was 46.1 ± 6.9 ng/sample, with a range of 29.1 to 70.8 ng/sample.

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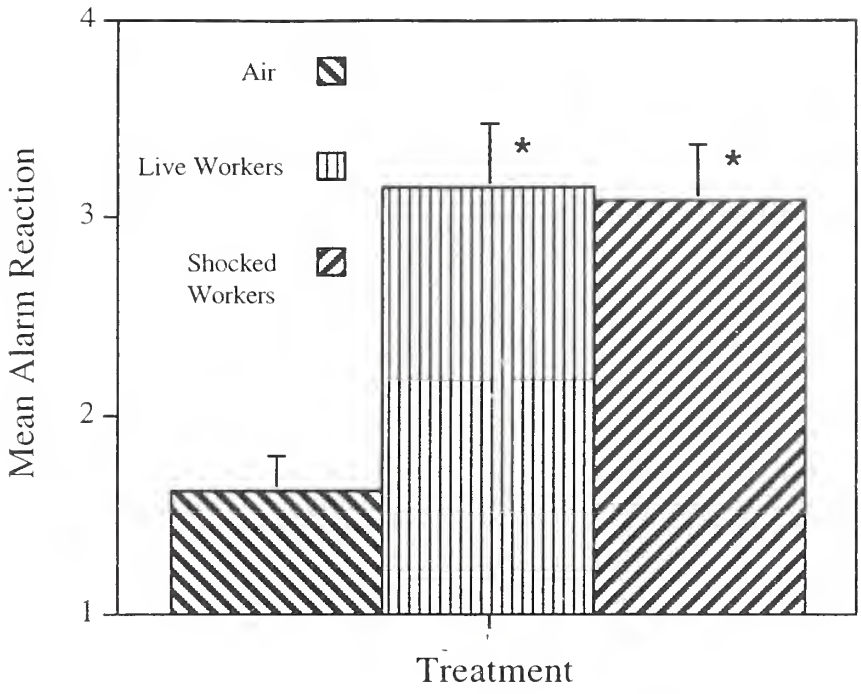

Figure 1. Comparison of the fire ant alarm reaction produced from control air, the air above disturbed live fire ant workers, and the air in vials in which fire ant workers were electrically shocked. * Significantly different from the air control ($P < 0.01$).

CRIS: 6615-32000-028-00D

IMPACT OF RED IMPORTED FIRE ANT TOXICANTS ON ENDANGERED INVERTEBRATE CAVE SPECIES

D.P. Wojcik and R.J. Brenner

Objectives: Assess susceptibility of endangered cave invertebrates to impact by fire ant and fire ant management practices and chemicals, and spatial foraging profiles near cave entrances in Texas.

Methods: As it is not feasible to test endangered cave species, cave crickets, a non-target scavenger, captured in caves in Texas were tested in two ways. First, cave crickets were allowed to feed on specific numbers of bait granules to determine the risk Amdro bait poses to the crickets. Second, cave crickets were allowed to feed on Amdro poisoned fire ant cadavers, to determine if the movement of toxicants via scavengers into the cave systems was possible. Additional tests were conducted using fish-bait crickets as surrogates for the cave crickets. The spatial distributions of foraging fire ants and foraging cave invertebrates were assessed at Camp Bullis, Texas, using dry oatmeal bait monitors placed after dark in a 9 by 4 grid surrounding the entrance of the cave. Foraging activity (presence / absence) was assessed in this 13 x 35 m area at 10 minute intervals for an hour. Spatial probability contours were developed to determine whether there was overlap between the two invertebrate species (ants and crickets).

Results: Amdro granules were toxic to cave crickets and fish-bait crickets feeding on bait granules. For females feeding on one granule the LT_{50} was 8 days and the LT_{90} was 10 days and for feeding on ten granules the LT_{50} was 5 days and the LT_{90} was 6 days. For males feeding on one granule the LT_{50} was 6 days and the LT_{90} was 8 days and for feeding on ten granules the LT_{50} was 5 days and the LT_{90} was 7 days. For females feeding on one dead ant the LT_{50} was 41 days and the LT_{90} was 84 days and for feeding on ten dead ants the LT_{50} was 22 days and the LT_{90} was 46 days. For males feeding on a dead ant the LT_{50} was 57 days and the LT_{70} was 85 days and for feeding on ten dead ants the LT_{50} was 21 days and the LT_{90} was 36 days.

Spatial analysis profiles (Fig. 1) clearly indicated overlap of foraging profiles, even after only 10 minutes. Therefore, it is possible that a uniform broadcast of Amdro, or other fire ant bait, may be consumed also by the non-target invertebrates. However, no research has been conducted to determine feeding preferences of these non-targets, and whether they would actually consume the fire ant bait granules, or whether the non-target invertebrates would consume dead or moribund fire ants in nature. These studies do indicate, however, the need to provide a toxic bait to fire ants at a time when fire ants are actively foraging, but when non-target vertebrates are not foraging. Precision targeting foraging ants, on the basis of both temporal and spatial foraging profiles may provide a suitable strategy. This research also illustrates the need for pesticide-reduced strategies involving biocontrol agents of fire ants, or non-toxic semiochemicals that may disrupt colony communication and subsequent survival.

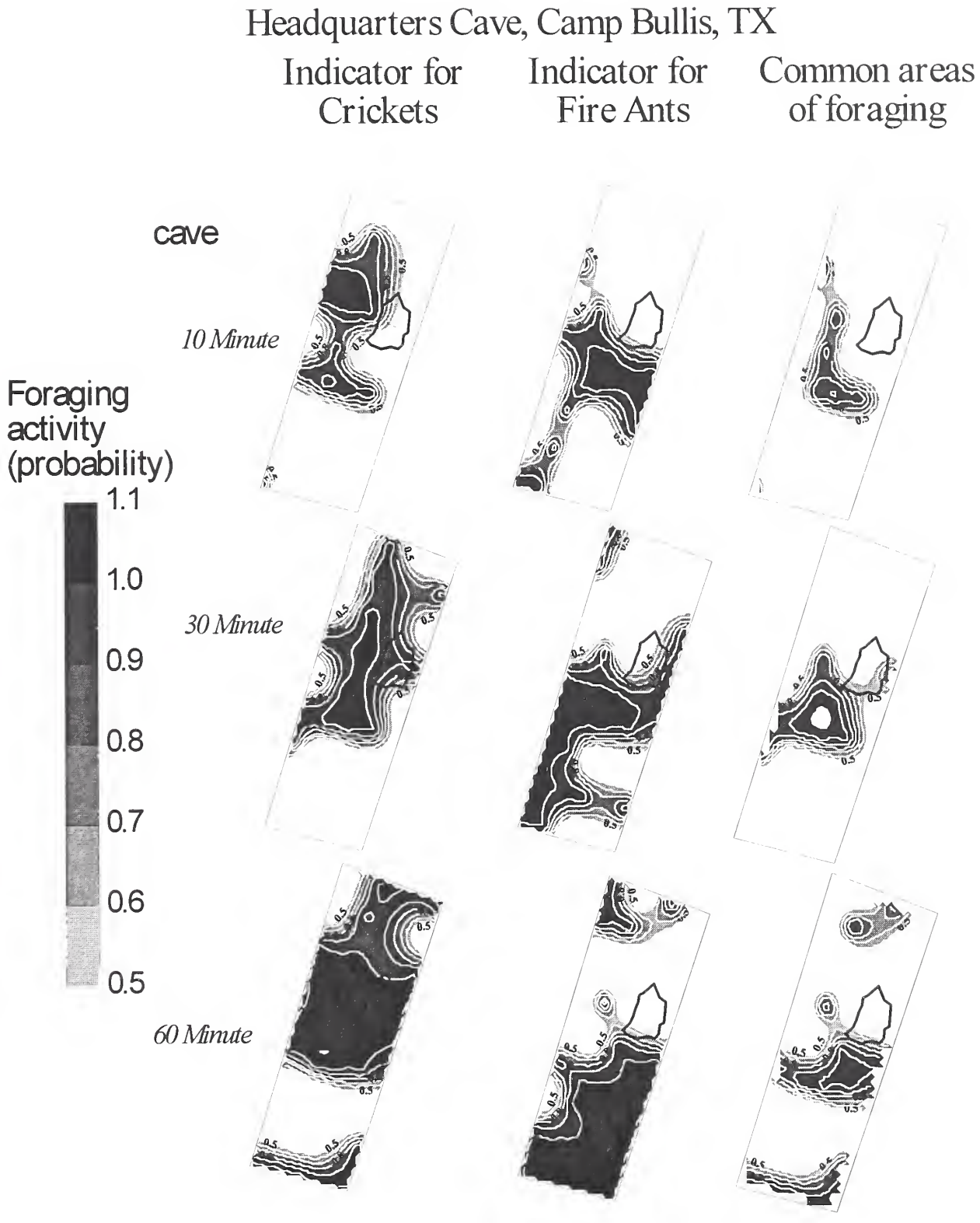

Fig. 1. Spatial patterns of foraging cave crickets and foraging fire ants, at selected time intervals, relative to a cave harboring the crickets. Each plot covers approximately a 13 x 35 m area. Contours show probability of crickets or ants finding dry oatmeal bait (4 by 9 grid) within designated time period.

CRIS: 6615-32000-029-00D

ABUNDANCE OF THE PARASITIC ANT, *SOLENOPSIS DAGUERREI*, IN ARGENTINA

J.A. Briano[1] and D.F. Williams

Objectives: To develop biological control agents for imported fire ants in the United States.

Methods: A total of 6,947 fire ant colonies were sampled in the provinces of Buenos Aires, Santa Fe, Entre Rios, Corrientes, Misiones, and Chaco for the presence of *S. daguerrei*. The sampling was conducted from April, 1987 to January, 1996. Colonies were sampled by cutting off the top of the mound with a shovel, allowing the host workers and adult parasites, if present, to move out of the mound. If the parasites were detected, the entire colony was excavated, returned to the laboratory and the ants separated by floating the ants out of the soil. The colonies were then put in rearing trays and the presence of adults and or queens of *S. daguerrei* was recorded. Most of the colonies were then shipped to the USDA-ARS-CMAVE at Gainesville and placed in quarantine for further research.

Results: In Argentina, *S. daguerrei* was detected in 10 sampling areas within the Provinces of Buenos Aires, Santa Fe, and Entre Rios in 1.4% to 6% of the colonies. The highest abundance of this parasite (6%) was found in the area of E. Flyn in Buenos Aires Province. Surprisingly, we did not find *S. daguerrei* in its type locality (Las Flores). The presence of adults and/or queens of S. daguerrei in the field is cyclic and in all areas surveyed, this parasite is in low densities. Large surveys such as this are not planned for the future and only surveys to find other locations of S. daguerrei populations for use in laboratory and field studies will be conducted. Several laboratory and field studies are underway with *S. daguerrei* and results of these studies will appear in future reports.

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CRIS: 6615-32000-029-00D

HOST-SPECIFIC ATTRACTION OF *PSEUDACTEON* FLIES (DIPTERA: PHORIDAE) TO FIRE ANT COLONIES IN BRAZIL

S.D. Porter

Objectives: Host specificity is an important issue that needs to be resolved before the introduction of exotic biocontrol agents. The objective of this study was to compare the host specificity of parasitic *Pseudacteon* decapitating flies to *geminata* and *saevissima* complex fire ants in the field in South America. The suitability of *saevissima* and *geminata* complex fire ants as hosts for *Pseudacteon* flies is an important biocontrol question because all native fire ants in the United States are in the *geminata* complex, while both of the imported fire ants in the United States are in the *saevissima* complex.

Methods: Three fire ant colonies in the *saevissima* complex were collected from the EMBRAPA, CNPMA research station in São Paulo State, Brazil. Three *Solenopsis geminata* colonies were collected from the CEPLAC research station in Bahia, Brazil. All test colonies lacked a mother queen and all sexuals were removed from the *S. geminata* colonies. Field tests were conducted at two sites near Araras in São Paulo State, Brazil (8-10 April 1996). At each site, the three *S. geminata* colonies and three *saevissima* complex colonies were set out in trays in four sequential tests. The numbers of active flies were estimated every 10 minutes during each test run. At the conclusion of these tests, all six colonies were returned to the lab and checked for pupating larvae.

Results: Parasitic *Pseudacteon* flies in Brazil showed a strong preference for fire ants in the *saevissima* complex. No *Pseudacteon* flies were attracted to three *Solenopsis geminata* colonies when they were set out in trays, but many flies were quickly attracted to three trays with *saevissima* complex colonies when they were set out between the *S. geminata* colonies. Even when both species of ants were placed together side by side, more than 99% of flies were over trays with *saevissima* complex ants. When all of the *saevissima* colonies were removed, leaving only the *S. geminata* colonies available, about 95% of flies ceased activity. Several flies, however, did transfer to the *S. geminata* colonies for a few minutes and at least one fly (*P. wasmanni*) attacked a few *S. geminata* workers. Altogether, 588 parasitized workers were collected from the *saevissima* complex colonies compared to 12 from the *S. geminata* colonies. Two hundred-sixty-two flies emerged from the *saevissima* complex colonies (50% *Pseudacteon tricuspis,* 39% *Pseudacteon litoralis*, 2.7% *Pseudacteon pradei,* 1.5% *Pseudacteon wasmanni*, 0.3% *Pseudacteon curvatus).* No adult flies emerged from the *S. geminata* colonies. These results demonstrate that *P. tricuspis* and *P. litoralis* are highly specific to *saevissima* complex fire ants and strongly indicate that they would pose little threat to native fire ants when they are released as biocontrol agents for imported fire ants in the United States. The other species were not sufficiently common to assess specificity.

CRIS: 6615-32000-029-00D

FIELD RELEASE OF *PSEUDACTEON* FLIES (DIPTERA: PHORIDAE) FOR CLASSICAL BIOLOGICAL CONTROL OF IMPORTED FIRE ANTS IN THE UNITED STATES

S.D. Porter and L. Alexandre N. DE SÁ

Objectives: The objective of this project is to develop techniques for successfully releasing the phorid fly *Pseudacteon tricuspis* in the field as a biocontrol agent for imported fire ants in the United States. Phorid flies in the genus *Pseudacteon* are promising classical biological control agents because their population-level impacts in South America have been sufficient to cause the evolution of a suite of fire ant defensive behaviors.

Methods: Permission for field release of this fly was obtained in June 1997 after more than two years of safety tests demonstrated that this fly was extremely host specific and posed no threat to people, animals, or plants. Release of this fly was also greatly facilitated by the development of techniques for rearing large numbers of these flies in our laboratory. Field releases of this fly began July 1997 at Kanapaha Botanical Gardens in Gainesville, FL. In September, we also began releasing flies at a dairy farm north of Gainesville and a site along Hogtown Creek to the west. So far, we have released about 800 flies at Kanapaha Gardens, 1200 at the dairy farm and 1500 along Hogtown Creek. About 30% of the flies were released as adults near disturbed fire ant colonies. These flies were allowed about 30 min. to mate in the lab prior to their release in the field. Adult flies were released in the hope that they would locate and attack fire ants in microhabitats best suited for their survival. The remainder of the flies were released as larvae in parasitized fire ant workers that were returned to their mother colonies after being attacked in the laboratory. Flies were released in parasitized ants so that we knew where they were located and that large numbers of ants had been parasitized.

Results: So far no flies have been recovered from Kanapaha Gardens, but we are continuing to monitor this site in case fly numbers are still too low to be easily detected. Seven flies were discovered at the dairy farm on October 21. This was the first confirmed field recovery of an introduced *Pseudacteon* fly in the United States. Six flies were also found at the Hogtown Creek site on October 24. We are very likely to find more flies at both sites in the next several weeks as they complete their 40-60 day life cycle. We should know by next spring if sufficient flies have survived to establish permanent populations at these two sites.

CRIS: 6615-32000-029-00D

STUDIES WITH THE MICROBIAL PATHOGEN, *THELOHANIA SOLENOPSAE* IN THE IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*, IN THE UNITED STATES

D.F. Williams, G.J. Knue and J.J. Becnel

Objectives: To develop biological control agents for imported fire ants in the United States.

Methods: Laboratory studies were conducted to try to infect fire ant colonies with spores of the microbial pathogen, *Thelohania solenopsae*. Small laboratory colonies (35) of *S. invicta* were presented with the spores of *T. solenopsae* in several formulations including sugar-water, honey-water, soybean oil, a paste made from insects, distilled water, and chicken egg yolk. All of the formulations were fed on by the worker ants. Several months following the introduction of the spore formulations to the colonies, all worker were checked for the presence of spores. To detect the percent of colonies infected, a sample containing 50-100 workers was ground in a glass tissue grinder with ca. 1 ml of water; one drop of the aqueous extract was examined by phase-contrast microscopy (400x) for the presence of spores.

Individual worker ants were examined for the presence of spores (meiospores and free spores) of the microsporidium by smearing the contents of their gasters on a microscope slide, adding a drop of water, placing a cover slip over the mixture and surveying under the microscope. In addition, Giemsa stains were prepared of the immatures stages of the fire ants.

Results: After several months, all of the workers were negative for the microsporidium. Also, vegetative stages of the microsporidium were not found in the larvae or pupae of the colonies by microscopic examination (400 and 1000x) of Giemsa-stained smears. Thus, none of the formulations + spores caused an infection of *T. solenopsae* in these laboratory studies Additional studies including both laboratory and field are underway to determine transmission of this microsporidium in colonies of the imported fire ant. In addition, studies to determine the effects of *T. solenopsae* on fire ant colony growth rates and affects on queens have been initiated.

MOSQUITO

AND

FLY

CRIS - 6615-32000-031-00D--Repellent Systems and Control Strategies for Mosquito/Vectors of Medical and Veterinary Importance

CRIS - 6615-32000-032-00D--Biological Control and Integrated Management of Bloodsucking and Nuisance Flies of Med/Ag/Vet Importance

CRIS: 6615-32000-031-00D

MEDIATION OF DEET REPELLENCY BY SPECIES, AGE, AND PARITY FACTORS IN MOSQUITOES

D.R. Barnard

Objective: Determine (1) the effect of combinations of species, age, and parity factors in mosquitoes on the protection period provided by deet and (2) the relationship between mosquito biting rates and oviparity at the time of repellent failure.

Methods: The responses of *Aedes aegypti*, *Anopheles albimanus*, and *An. quadrimaculatus* to deet were determined by testing 5, 10, 15, and 20 day-old nulliparous females and 10, 15, and 20 day-old parous females. Adult mosquitoes were held in stock cages at 75% RH and 26°C and provided sucrose and water *ad libitum*. Parous females were obtained by membrane-feeding 5 day-old mosquitoes on bovine blood then providing water for oviposition 72 h later. All mosquitoes were selected for testing based on a positive pre-test response to human host stimuli. Repellent Test Procedure: One ml of ethanolic 25% deet was applied to the forearm of a human volunteer (coverage area = 650 cm^2). The volunteer placed their arm inside a cage containing 100 mosquitoes for 3 min and observed for mosquitoes that landed and attempted to feed on the treated skin surface. This procedure was repeated for each test cage at 60 min intervals until 2 or more bites were observed in a 30 minute period in each cage. Complete Protection Time (CPT), in h, and percentage biting were the responses recorded. A split plot design was used with whole units completely randomized. Main plots were mosquito species, subplots were mosquito age and mosquito parity. There were two levels of parity at each level of mosquito age except for 5 day-old females. The same human subject was used in all tests.

Results: Complete Protection Time for deet was shortest against *An. albimanus* (1.6 h) and *An. quadrimaculatus* (1.5 h) and longest against *Ae. aegypti* (6.5 h). Differences within species were not influenced by mosquito age or parity or their interaction. These results suggest that the CPT of deet under field conditions would be similar regardless of the age or parity structure of the biting mosquito population. This finding is important because parous mosquitoes, which may be infectious, are the logical target of personal protection measures in disease-endemic areas. Percentage Biting responses were highest in *An. albimanus* (14.2%) followed by *An. quadrimaculatus* (7.0%) and *Ae. aegypti* (2.9%) and were twice as high in parous females (10.8%) as in nulliparous females (5.9%). There was significant interaction between mosquito species and parity and between parity and mosquito age. Mean percentage biting was significantly different between nulliparous (1.6%) and parous 20 day-old *An. albimanus* (35%). When compared by mosquito age within parity group, there was no difference in mean percentage biting between different aged parous or nulliparous *Ae. aegypti* or *An. quadrimaculatus* whereas biting rates decreased significantly in 20 day-old nulliparous *An. albimanus* (0.2%), compared with younger females. In contrast, mean percentage biting in parous *An. albimanus* increased with mosquito age.

CRIS 6615-32000-031-00D

GC/MS ANALYSIS OF HUMAN SKIN EMANATIONS BY THERMAL DESORPTION OF VOLATILES FROM HANDLED GLASS BEADS

U.R. Bernier, M.M. Booth[1], R.A. Yost[1], D.L. Kline, D.R. Barnard and C.E. Schreck

Objective: Develop a method for the identification of human skin emanations that attract the Yellow Fever mosquito *(Aedes aegypti)*. The method should minimize water collection, minimize the possibility of sample loss, minimize solvent or adsorption effects, and concentrate compounds that attract *Aedes aegypti*.

Methods: Five to twelve 2.9 mm diam. glass beads are rubbed in the palms of the hands or against the skin surface for 15 min. A fritted injection port insert is placed in the injection port reversed or upside-down compared to normal operation. The glass beads fit directly into the gas chromatograph injection port insert of a Varian 3400 gas chromatograph. The beads are held in place by the frit and the column entrance is unobstructed, below the frit. The injection port is sealed off by an unused septum and heated ballistically to 250 C to allow thermal desorption of volatiles from the glass beads. The volatiles are cryo-focused with liquid nitrogen in the the first 50 cm of column. A nonpolar HP5 capillary column, as well as a polar HP-FFAP are routinely used to effect separation. The chromatograms from an analysis using a 25 m x 0.20 mm i.d. HP-FFAP column are shown in Figure 1. After the 10 min cryo-focusing collection period, the oven is ramped from 40°C to 236°C at rate of 11°C/min, followed by a 26 min hold at the final temperature. The mass spectrometer is scanned in either pulsed positive ion negative ion chemical ionization (PPINICI) mode or electron ionization (EI) mode.

Results: Handled glass collects human skin emanations which are attractive to *Aedes aegypti* mosquitoes. Emanations from humans contain a significant amount of water vapor; therefore, direct analysis of this sample is detrimental to glass capillary columns. Alternative methods of sample collection can be performed by washing the skin with solvents or by concentrating volatiles onto an adsorbent phase. It is desirable to avoid these methods since the possibility exists that they may artificially introduce uncertainty into the amounts collected or possibly not allow the collection or identification of some compounds. The use of small glass beads allows the collection of volatiles which produce mosquito attraction and minimizes the deposition of water. Chromatograms from different human subjects were similar with respect to compounds present but differed in the amounts of these compounds (Figure 1). Employing this method has led to the identification of over 400 compounds emanating from human skin. Although the major components in the chromatograms are carboxylic acids, some of the minor components attracted *Aedes aegypti*.

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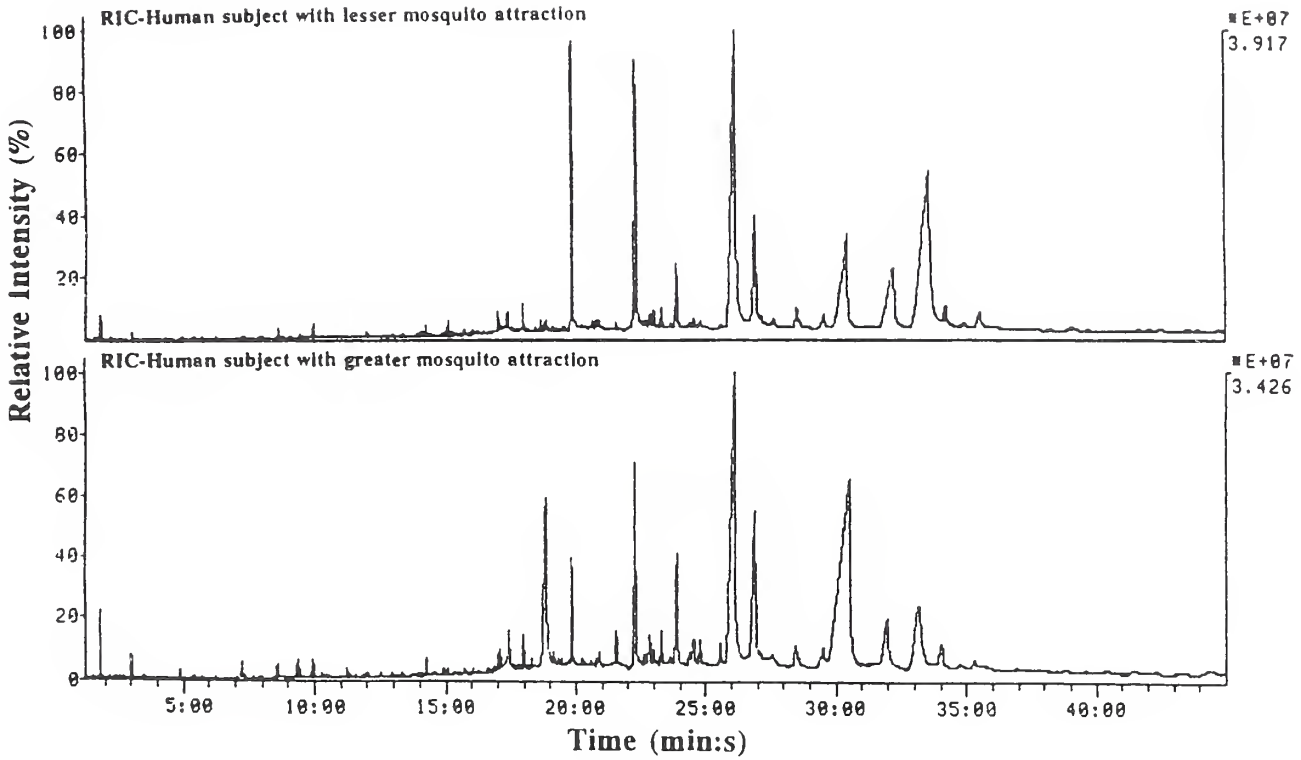

Figure 1. Comparison of reconstructed ion chromatograms (RICs) for two human subjects who differ markedly in attraction of mosquitoes. Mass spectrometry was conducted in electron ionization (EI) mode and separation was effected by a 25 m x 0.20 mm i.d. HP-FFAP capillary column. The peak at 18.8 min found in the RIC of the more attractive host has been identified as glycerol, originating from a hair product used by the subject. Lactic acid is found at 17.3 min and the highest peak in the chromatograms is 9-hexadecenoic acid at 26.0 min.

CRIS 6615-32000-031-00D

AN. QUADRIMACULATUS SPECIES CL HATCHING FACTOR

D.A. Carlson, U.R. Bernier and G.E. White

Objective: Isolation and identification of *An. quadrimaculatus* Species C I Hatching Factor. Discovery of the chemicals involved could explain the mechanism of egg hatch in this floodwater mosquito species and result in a novel method of mosquito control that is environmentally safe.

Methods: Aliquots of extracts and fractions were isolated from swamp water and swamp soil spotted on filter paper and dried, then added to mosquito eggs in 25 ml distilled water in small beakers. Active fractions, but not fresh, tap, or distilled water, cause hatching of eggs within a few minutes of flooding. Swamp water (IOOL) from the Suwannee River basin was extracted with hexane solvent and the crude extract fractionated on a silica gel column to give an active fraction that eluted with hexane/20% ether. A hexane extract of swamp soil was similarly fractionated by high performance liquid chromatography, using 70% methanol in water, and showed retained biological activity.

Results: The odorous yellow oils obtained by fractionation of soil extract contain many compounds as shown by TLC. GC-MS of swamp soil and of silica gel column fractions showed identifiable terpenes including camphor and camphor-related compounds. The fractions were checked for the presence of several soil-borne compounds reported in the literature including 2-methyoxypyrazine, methyl isoborneol and geosmin. Small amounts of the latter two were observed by GC-MS in the polar fractions. Separation and isolation steps will be repeated during Spring 1998 when mosquitoes become available again.

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PEPTIDE HORMONE MIMICS OF TRYPSIN-MODULATING OOSTATIC FACTOR

D.A. Carlson and L. Okedi

Objective: Develop new species-specific methods of insect control based on peptide hormones that use natural, biorational routes to interfere with egg development, particularly for bloodfeeding insects. This discovery could lead to novel control methods for blood-feeding insects near cattle-rearing facilities, where area treatment, attractive traps, or the use of autocidal methods may be deployed.

Methods: Injected and topically applied peptide mimics of TMOF into living blood-fed stablefly and mosquito females interfered with synthesis of egg yolk intended for immature eggs, resulting in eggs which never mature. Several new cooperators were involved, with compounds being synthesized specifically for this effort by G. Garcia (Walter Reed Army Institute of Research), Al Chung (U. Florida Protein Core), and R. Nachman (College Station, TX, ARS).

Results: Six novel non-peptide mimics of peptide hormones based on patented TMOF peptide hormones were obtained by synthesis, then showed to have potent sterilizing activity on mosquitoes and stable flies over a long period corresponding to normal egg development time. Trypsin-like enzyme synthesis is inhibited with 5 microgram treatments per female. As the blood meal remains undigested or poorly digested, egg yolk protein does not appear in the ovaries and the eggs will not mature or hatch. The egg development was followed for 7 days with adult female stableflies and 5 days with female *Aedes aegypti* mosquitoes after they were injected or treated with Trypsin-Modulating Oostatic Hormone (TMOF) related compounds dissolved in saline or DMSO/acetone, respectively. Inhibited egg development was observed, along with some toxic effects from some of the peptides. Dose-response studies and quantitative trypsin determination in treated insects are still underway with these compounds.

CRIS: 6615-32000-031-00D

EVALUATION OF *BEAUVERIA BASSIANA* (MONILIALES: MONILIACEAE) AGAINST THE LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (COLEOPTERA: TENEBRIONIDAE) IN POULTRY LITTER, SOIL AND AN INFECTIVE PUPAL TRAP

C.J. Geden, J.J. Arends[1], D.A. Rutz[2] and D.C. Steinkraus[3]

Objectives: Litter beetles, including the lesser mealworm (*A. diaperinus*) are serious structural pests of poultry houses throughout the world. The beetles are exceedingly difficult to control with chemical insecticides. The objective of this study was to evaluate the entomopathpgenic fungus *Beauveria bassiana* as a biological control agent against this pest.

Methods: Serial dilutions of four strains of *B. bassiana* were tested against adult and larval beetles in forced-contact bioassays using liquid and starch dust formulations. Two of the strains (HF88 and HF89) were originally isolated from house flies; the other strains, (WV and NC) were isolated from *A. diaperinus* during local epizootics of *B. bassiana*. Assays in treated poultry litter and soil were conducted by adding mature beetle larvae or adults to plastic cups containing 150 cm^3 of turkey litter or soil treated 24 h later with 5 ml of an aqueous suspension or 1 g of starch dust containing *B. bassiana* conidia. Beetle mortality was determined by removing and counting live beetles remaining in the cups 2 weeks after treatment. Finally, an infective pupation trap was tested by allowing beetle larvae to enter and pupate in cardboard traps treated with *B. bassiana* conidia.

Results: In forced contact bioassays, young larvae of the lesser mealworm were highly susceptible to a strain of the fungal pathogen *Beauveria bassiana* (WV) that was isolated from a natural epizootic in *A. diaperinus* (5-d-old larvae $LC_{50}=1.73x10^2$ conidia/ml, $LC_{90}=9.86x10^3$; 10-d-old larvae $LC_{50}=2.49x10^2$, $LC_{90}=4.74x10^4$,). Adult beetles were approximately 1000 times less susceptible than young larvae ($LC_{50}=1.94x10^5$, $LC_{90}=6.41x10^6$). Mature larvae and adult beetles were more susceptible to WV and to a second beetle-derived strain (NC) than they were to two strains that originated in house flies (HF88 and HF89). Starch dust formulations were more effective than aqueous suspensions when conidia were applied to poultry litter containing manure, chicken feed and pine shavings. Soil treatments with corn starch containing the WV and NC strains provided 100% control of beetle larvae at $2.5x10^{11}$ conidia/m^2; aqueous suspensions were less effective. An infective pupation trap consisting of conidia-treated cardboard provided 100% control of beetle larvae at 4 x 10^5 conidia/cm^2 of trap surface. It is estimated that $3x10^{11}$ conidia could be used to treat a 1200-m^2 poultry house with infective traps.

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IDENTIFICATION OF *MUSCIDIFURAX* SPP., PARASITOIDS OF MUSCOID FLIES, BY COMPOSITION PATTERNS OF CUTICULAR HYDROCARBONS

C.J. Geden, U.R. Bernier, D.A. Carlson, and B.D. Sutton

Objectives: In a companion study in this report, Bernier *et al.* demonstrated that pooled samples of *Muscidifurax* spp. house fly parasitoids possessed species-specific cuticular hydrocarbon patterns. The objective of this study was to extend this application and determine the practical utility of hydrocarbon analysis as a taxonomic tool for identifying parasitoids.

Methods: For analysis of individual specimens, *M. zaraptor* were obtained from a culture originally established from cattle feedlots in Nebraska. *M. raptor* were collected in the field from wild house fly pupae from a Florida dairy farm. Specimens from three colonies of *M. raptorellus* were obtained from J. J. Petersen, (ARS, Lincoln, Nebraska), and included a Peruvian solitary culture, a Chilean gregarious culture, and a culture of Nebraska gregarious parasitoids. Ten females were analyzed for each species and isolate. For evaluation of geographic variation within *M. raptor*, groups of 20 females were examined by removing voucher specimens from parasitoid colonies that were established from a poultry farm in Florida (CMAVE culture no. 1250, specimens from F23) and from livestock farms in Germany (no. 1201, F113), Hungary (1215-B, F54), France (1226, F30) and Brazil (1220-A, F31) in 1988-1992. Parasitoids in the voucher material were removed from the colonies at random and were of mixed age at the time of collection. Specimens were washed and analyzed as described in the summary by Bernier *et al.*

Results: Species-specific patterns of cuticular hydrocarbons allowed identification of *M. raptor, M. zaraptor* and *M. raptorellus.* A total of 18 components, all C29-C37 alkanes and methylalkanes, accounted for over 90% of the total hydrocarbons for all three species. *M. zaraptor* was characterized by a high ratio (11.9) of 3-MeC31:internal Me2C35's, whereas this ratio was < 3 for the other species. *M. raptorellus* was characterized by a low (<1) 3-MeC31: 3,7,15-Me3C37 ratio compared with ratios of 3.1 and 6.3 for these components in *M. raptor* and *M. zaraptor*, respectively . Three populations of *M. raptorellus* could be distinguished based on two other component ratios (5- and 7-MeC31: 3MeC32, 5- and 7MeC31:3,7- to 3,15-Me2C33) with either 100% (Nebraska population) or 90% (Chilean and Peruvian populations) certainty. Comparison of *M. raptor* colonies established from 5 different locations (Florida, France, Germany, Brazil, Hungary) indicated that the hydrocarbon pattern was highly conserved in this species. A dichotomous key to species based on ratios of cuticular hydrocabon components unambiguously classified the 50 samples of *Muscidifurax* spp.used to construct the key, plus 5 additional samples from different geographic locations. A manuscript on this work has been submitted for publication in the journal "Biological Control".

CRIS: 6615-32000-031-00D

EVALUATION OF AMERICAN BIOPHYSICS COUNTER FLOW TECHNOLOGY MOSQUITO TRAP

D.L. Kline

Objective: New trapping technology is needed if removal trapping is to become a viable option in the development of non-insecticide based integrated mosquito management programs. This study was conducted to determine the efficacy of several traps developed using a new counter flow technology and compared with the efficacy of conventional mosquito traps.

Methods: The American Biophysic model AB-PRO trap was compared with the counter flow (designated as PJ) trap for their ability to capture mosquitoes. Each trap was supplied with 500 cc/min CO_2 from a compressed gas cylinder and an octenol slow-release package. Traps were run for 12 nights in an wooded area adjacent to protected wetlands. Trap types were alternated between positions each night. Data were analyzed using T-tests.

Results: Although baited exactly the same, the PJ trap type caught 7.3x as many mosquitoes (4630 vs 631) as the AB-PRO trap. The *t* statistic (4.5, 22 df) was highly significant (Prob>|T| 0.0002). Each trap type caught 14 species of mosquitoes, the composition of which differed by only 1 species for each trap type. Differences in trap collections are attributed to trap geometry. The PJ trap mixtures the CO_2 with surrounding air so that it is not released at the 100% concentration level. We believe that this dilution effect allows the mosquitoes to follow the odor plume closer to the trap. The counter flow principle also results in less turbulence of the air near the trap, which causes the mosquitoes to more closely approach the trap entrance and to be drawn into the trap.

CRIS: 6615-32000-031-00D

EVALUATION OF LIMBURGER CHEESE AND WORN SOCK AS SOURCE OF ATTRACTANTS FOR *AEDES AEGYPTI* IN AN OLFACTOMETER

D.L. Kline

OBJECTIVE: Discover and identify new mosquito attractants for use in baited traps for removal trapping of mosquitoes.

METHODS: To determine if Limburger cheese and/or socks worn by human host produced volatiles which would elicit an upwind orientation response in 6-8 day old laboratory-reared, host-seeking *Aedes aegypti*, a dual-port olfactometer was used. The olfactometer consisted of three stacked chambers (35 cm high x 90 cm long x 48 cm wide). Only one chamber at a time was used for assays. Outside air was conditioned prior to entry through the choice ports, the mosquito trap, and the olfactometer by passing through a series of charcoal filters and then heated and humidified (if necessary). Conditions in the olfactometers were 27°C, 60% relative humidity with an air flow of 1 liter/sec. One hour before initiation of tests ca. 75 females were placed into the olfactometer chamber and allowed to acclimate. Treatments were placed into two test ports upwind of the traps and olfactometer chamber. For each olfactometer test, one port contained the treatment (Limburger cheese, worn sock, or human arm) and the other port was used as an untreated control. After 3 minutes, the numbers of mosquitoes in the treatment and control traps were compared. Ten tests were run with each treatment.

RESULTS: Limburger cheese resulted in a mean response of 6.4% vs 0.0% for the check; the worn sock resulted in a 66.1% mean response vs 0.1% for the check; and the human hand resulted in an 80.1% mean response vs 0.1% for the check. Presently, we have identified 25 volatile compounds from the worn sock for which testing in the olfactometer has been initiated. The worn sock is also being evaluated against natural populations of woodland mosquitoes.

CRIS: 6615-32000-031-00D

AN ANALYSIS OF THE *ANOPHELES (ANOPHELES) QUADRIMACULATUS* COMPLEX OF SIBLING SPECIES (DIPTERA: CULICIDAE) UTILIZING MORPHOLOGICAL, CYTOLOGICAL, MOLECULAR, GENETIC, BIOCHEMICAL, AND ECOLOGICAL TECHNIQUES IN AN INTEGRATED APPROACH

J.F. Reinert and J.A. Seawright

Objective: To provide taxonomic keys for the five members of the *Anopheles quadrimaculatus* complex. This report is the last on a long-term project on this important species complex. Historically, the members of this complex were the principal vectors of malaria and also important pests in the eastern half of the United States. About ten years ago, the existence of the species complex was detected. Which of the five species was most important in vectoring malaria is not known.

Methods: The *Anopheles quadrimaculatus* complex of 5 cryptic species was analyzed utilizing multiple techniques that included morphological, cytological, molecular, genetic, biochemical, and ecological procedures. Isofemale broods were reared and prepared for analysis by standard taxonomic techniques used for mosquitoes. Each brood was initially assigned to one of the five known species by analysis of the isozyme pattern in the adult females. All life stages (egg, 4th-instar larva, pupa, and female and male adults) were described using morphological features and pertinent stages or structures were illustrated.

Results: Binomial names were assigned to the four new species of the complex. These names will be released after final publication of the descriptions. A neotype for *An. quadrimaculatus* was designated and the synonymy of *An. annulimanus* Van der Wulp was confirmed. Several new morphological features, not previously included in descriptions of anopheline mosquitoes, were studied and described, and keys were assembled for morphological characters for the eggs, 4th-instar larvae, pupae, adult females and male genitalia. Also, a key for the isozymes of the 5 species was developed, and a cytogenetic comparison of the polytene X chromosomes from salivary glands was done. A monograph was written and in addition to the morphological analysis and associated keys, we included a summary of other information dealing with pertinent bionomics data on the immature and adult stages, maps on the geographic distribution for each species, and the genetics and cytogenetics of the five species.

CRIS: 6615-32000-032-00D

BIODEGRADATION OF POULTRY MANURE BY HOUSE FLY (DIPTERA: MUSCIDAE)

D.R. Barnard, R.H. Harms[1], and D.R. Sloan[1]

Objective: To assess the nature and extent of physical changes in poultry manure that result from feeding activity by house fly larvae (Musca domestica) and to characterize fly developmental responses to manure parameters.

Methods: In a series of three experiments we determined (1) the relationship between larval density and changes in manure parameters; (2) the effect of larval density on manure degradation and fly growth and survival when the manure medium consisted of (a) 100% fresh manure, (b) different concentrations of fresh and degraded manure, and (c) 100% degraded manure; and (3), the relationship between larval density and manure degradation in a simulated laying hen environment. A completely randomized design was used in the first and third experiments (100, 300, 600, and 900 eggs/100 g manure; 300, 600, 900, and 1200 eggs/100 g manure, respectively) and a 4 × 5 factorial design was used in the second experiment (Factor 1: 100, 300, 600, and 900 larvae/100 g manure; Factor 2: 0, 25, 50, 75, and 100% fly-degraded manure). All treatments were replicated 3 times.

Results: In experiment 1, reductions in manure mass and moisture content were greatest at larval densities of 600/100 g manure and dry matter decrease was greatest at 300-900 larvae/100 g manure. In experiment 2, larval feeding reduced manure mass and moisture content the most in 100% fresh manure and the least in 100% degraded manure. In the simulated field study, manure mass decreased least at the lowest larval density but changes in moisture and dry matter content were not influenced by the numbers of feeding larvae. In all experiments, larval survival and pupal mass both were inversely proportional to larval density. Three main conclusions were drawn from this study: (1) larval density influences change in manure parameters only when the quantity of developmental medium is limited; when larvae have access to fresh manure, change in manure parameters is unaffected by densities up to 1200 larvae/100 g manure/day. (2) Two successive generations of flies can be reared on the same aliquot of manure. In such cases, larval survival ranged from 0.7% to 62.2% and pupal mass ranged from 5 to 7 mg. (3) Under simulated field conditions, 1200 larvae/100 g manure/day resulted in a 75% reduction in manure mass, a 90% reduction in moisture content, and a 35% reduction in dry matter content. These changes are accompanied by 17% larval survival and pupae with mean weights of 5.7 mg.

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CRIS: 6615-32000-032-00D

ELIMINATION OF A FIELD POPULATION OF *AEDES AEGYPTI* IN FLORIDA BY INTODUCTION OF THE PATHOGEN *EDHAZARDIA AEDIS*

J.J. Becnel

Objective: To challenge a field population of *Aedes aegypti* in Florida with the parasite *Edhazardia aedis* and measure the impact of the infection on mosquito density.

Methods: This was the second year of tests conducted in a 10 X 30 meter screened enclosure located in Gainesville Fl. Containers from the previous year study (as described below) were left in place over the winter and flooded in April 1997 to initiate the *Ae. aegypti* population. As of April 30, 1997, *Ae. aegypti* larvae were present in all containers. Four rows of golf cart tires (26 total) were positioned on racks 1 meter above the ground. One gallon plastic containers were placed into each tire to which autoclaved leaves and 1 liter of well water were added. Paper strips (19 X 12.5 cm) lined each container to provide for oviposition. Two rabbits in 1 X 3 meter cages were located in opposite corners and served as a blood source. Containers were treated on July 17, 1997 as follows: One container was randomly selected from each row to serve as covered controls. Two of these were contaminated with 25 infected *Ae. aegypti* larval equivalents (3.2 X 10^7 spores) and two with 25 healthy *Ae. aegypti* larval equivalents. All open containers were contaminated with 25 infected *Ae. aegypti* larval equivalents and left uncovered. Pupae were removed from the covered containers daily, set up individually in vials for emergence and held for 48 hours. The infection status of each adult was determined by the presence of spores. Egg papers were collected weekly from the open containers and cut in half; one half was returned to the container and flooded, the other half hatched in the laboratory, reared to the fourth instar and examined for vertical infection. The number of immatures in each container were determined weekly beginning July 17, 1997 and the number of patent infections (either vertically or horizontally induced) recorded.

Results: Unexposed control containers (combined) had a total of 410 larvae at the start of the test from which 171 adults emerged (58% mortality). Exposed containers (combined) had a total of 361 larvae present from which 21 adults emerged (94% mortality). Seventy percent of the female adults that emerged from the exposed containers were infected with *E. aedis*. Mosquito densities were high during the period prior to treatment as measured by direct count and eggs sheet data (Fig. 1). At the time of exposure, there were approximately 150 larvae per container (Fig. 1). Infections of *E. aedis* were first detected in larvae 2 weeks post-exposure. By week 5, 70% of larvae were infected (horizontal + vertical) and 7 weeks post-exposure 82% of larvae were infected (Fig. 2). Infection levels in female adult mosquitoes were also high as determined by the presence of vertically infected progeny from eggs sheets, which by week 6 reached 70% and by week 7 60% (Fig. 2). Few larvae or adults were present after week 9 (Fig. 1), but all individuals present were infected (Fig. 2). The mosquito population was completely eliminated by 11 weeks post-exposure, at which point no containers contained immatures (Fig. 3) and oviposition activity ended.

Fig. 1

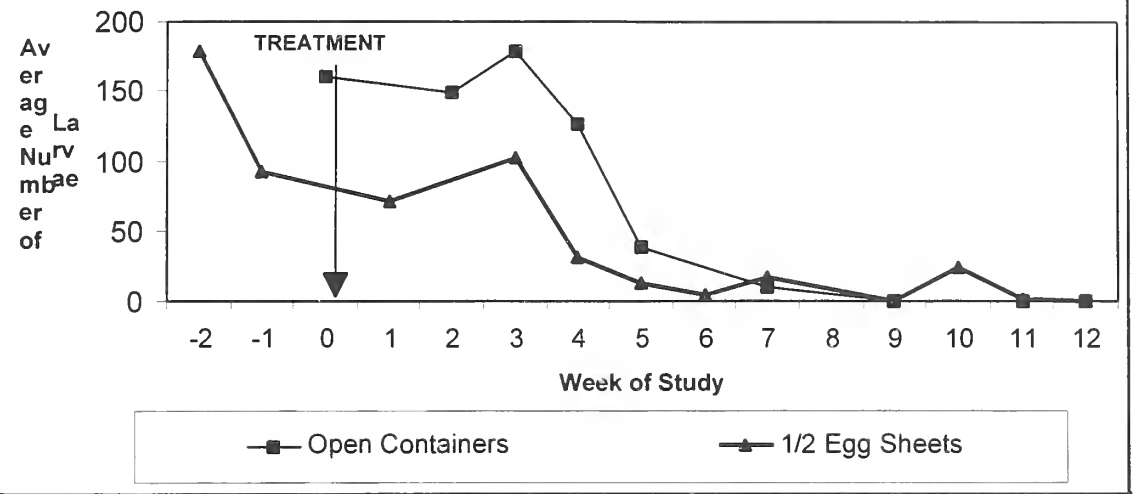


Fig. 2

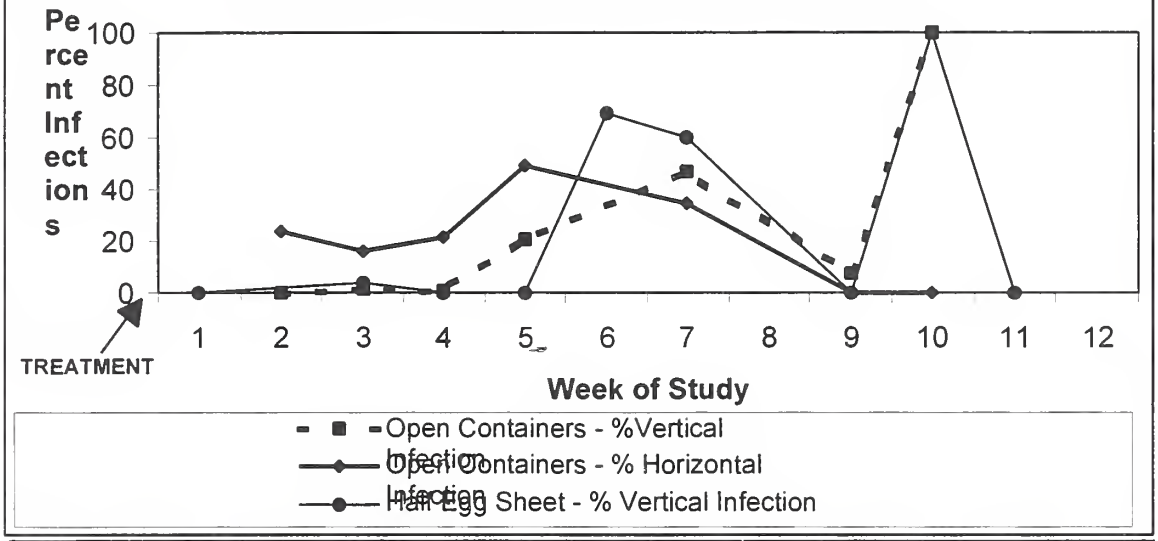
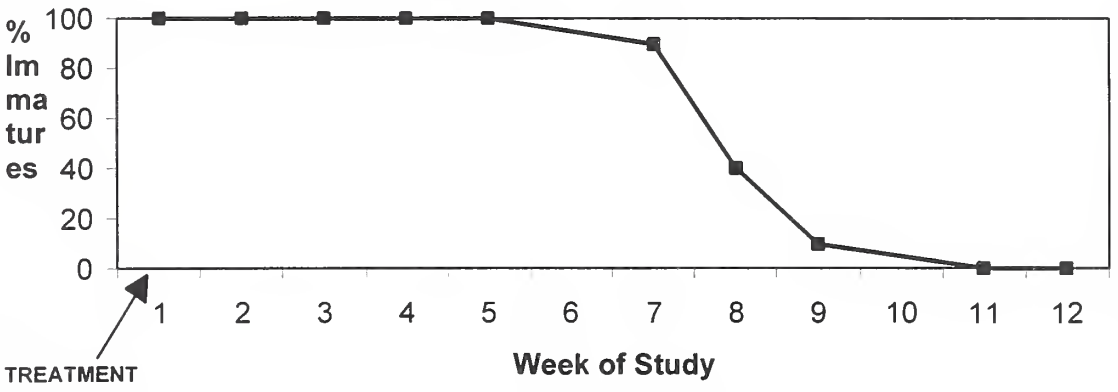


Fig. 3



CRIS: 6615-32000-032-00D

HYDROCARBONS FROM PARASITIC WASPS OF THE GENUS *Muscidifurax*

U.R. Bernier, D.A. Carlson and C.J. Geden

Objective: Establish a chemical method, using gas chromatography (GC) and mass spectrometry (MS), for species identification of *Muscidifurax*. The method should allow for determining colony cross-contamination and possibly identifying species prior to emergence.

Methods: *M. uniraptor*, *M. raptor*, *M. raptorellus*, and *M. zaraptor* were collected from various North American locations. The surface hydrocarbons are collected by hexane-washing of 20 wasps from each species and sex. The hydrocarbons are analyzed by GC and the chromatograms examined for differences. GC/MS was used to identify the compounds present. Samples are injected onto a 30 m x 0.20 mm i.d. DB-1 capillary column via a cold on-column injector. The column ramp consists of a 2 min hold at 60°C, followed by a 10°C/min ramp to 220°C, a second ramp of 3°C/min to 310°C, and a hold at that temperature to elute all components. The mass spectrometer is operated in electron ionization (EI) mode with tune parameters chosen to increase the transmission of ions in the m/z 200-600 range relative to the m/z 50-200 range.

Results: These parasitic wasps are often difficult to distinguish by conventional taxonomic techniques. Molecular techniques such as mitochondrial DNA can discriminate between these species; however, differentiation of *M. uniraptor* and *M. raptorellus* is difficult using this technique. Species within the genus *Muscidifurax*, as well as the sex, can clearly be differentiated by examining the gas chromatograms of the cuticular hydrocarbons. Identification of the alkanes by mass spectrometry shows that similar 11-methylene interrupted dimethylalkanes and 3/7-methylene interrupted trimethylalkanes are present for all members of the genus except for *M. uniraptor*. Methylalkanes with this interruption pattern are rare compared to those found on insects reported in the literature, but are found in significant amounts in these insects. Additionally, sexual dimorphism is observed in long chain methylalkanes (C_{21}-C_{39} backbones) present on male and female cuticular surfaces for these species. Females have methyl branches located externally on the carbon chain for dimethyl-, trimethyl-, and tetramethylalkanes, whereas males have dimethyl- and trimethylalkanes located internally on the hydrocarbon backbone chains. Example mass spectra are provided in Figure 1, where the 3/7-methylene interruption pattern (a characteristic of Muscidifurax) is observed in both sexes; however, the location of the methyl branches on the pentatriacontane backbone displays the sexual dimorphism.

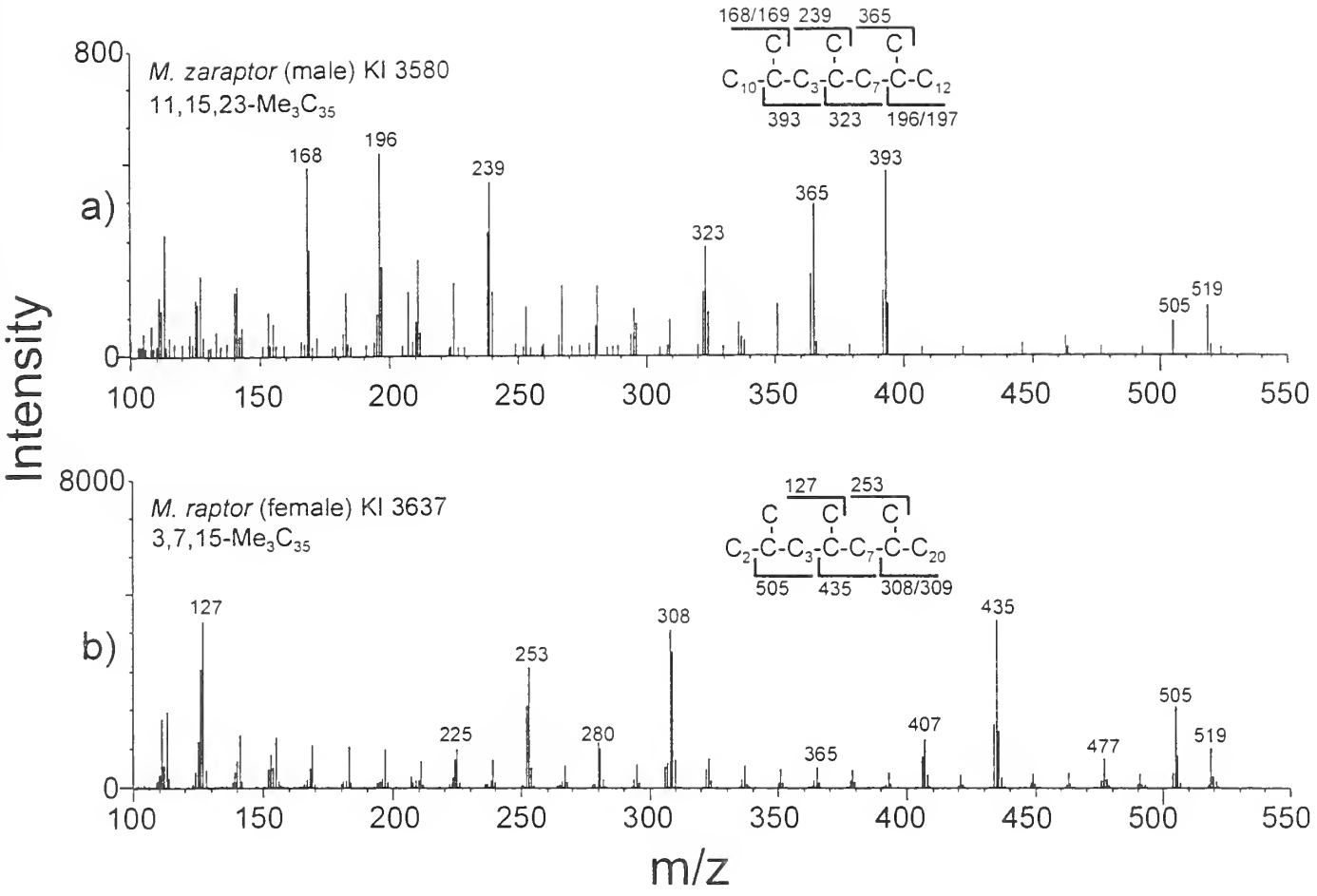


Figure 1. Mass spectra of the predominant trimethylalkanes observed in a) males and b) females of *Muscidifurax*. Males are characterized by internal 3/7-methylene interrupted trimethylalkanes, such as 11,15,23-trimethylpentatriacontane, whereas females have externally-branched 3,7,15-trimethylpentatriacontane.

CRIS 6615-32000-032-00D

SEX AND AGGREGATION PHEROMONES IN FILTH FLIES RELATED TO *Musca domestics.*

D.A. Carlson and U.R. Bernier

Objective: Efforts with U.S. Armed Forces entomologists overseas continued in an ongoing study of the sex and aggregation pheromones of the common house fly and related muscid fly species. Control or reduction of filth-breeding flies is the desired objective during deployment of U.S. Armed Forces to the Middle East and around the Pacific Basin; however, the efficacy of baits against species of filth flies encountered in the Middle East and Asia is unknown.

Methods: In a cooperative effort with LCDR Stan Cope and LCDR G. Tetreault, collections of filth-breeding Muscidae were made by military entomologists in Egypt, Zanzibar, Kenya, Viet Nam, Guam, and Australia. The use of 99-well plastic immunoplates for shipping the flies precluded the need to pin insects in the field, and brief drying before shipment prevented loss of hairs or heads. After we obtained shipments, they were catalogued and sent to A. Pont (UK) who identified the flies as *M. d. domestics, M. d. calleva, or M. d. curviforceps;* or as *M. sorbens, M. biseta, M. ventrosa or M. vetustissima* (the Australian Bush fly).

Results: About 450 hydrocarbon profiles were obtained, and GC-MS work has been completed on representative specimens. It appears that some housefly strains from Egypt, Okinawa and Viet Nam look very much like USDA colony flies, but others do not. *Musca d. domestics s.str.* have been identified from Egypt, with the precise location of each collection documented by GPS satellite readings recorded by military entomologists. *Musca d. curviforceps* females appear quite different from *Md. domestics. Musca domestics s.* 1. are dominated by (Z)-9-isomers from 23 to 37 carbons, while *M. sorbens and M. vetustissima* possess a wide distribution of positional isomers, including the 9-, 10-, 13-, 14-, 15- and 16-tritriacontenes. Novel sets of alkenes and dienes found in *M. biseta* are similar to those in *M. sorbens and M. vetustissima,* and females contain larger amounts of alkenes than males with the double bonds variable but located nearer the center of the chains. Improvements to baits by the addition of novel synthetic alkenes as pheromone candidate components is possible but much of the composition of sex or aggregation pheromones in the other species remains to be determined. However, these alkenes can be readily identified with available knowledge and equipment using dead, dried flies.

CRIS 6615-32000-032-00D

THE SUSCEPTIBILITY OF THE MOSQUITO *CULEX NIGRIPALPUS* TO THE NEMATODE PARASITE *STRELKOVIMERMIS SPICULATUS*

T. Fukuda, S.E. White, O.R. Willis and D.R. Barnard

Objective: To determine the susceptibility of *Culex nigripalpus*, the vector of St. Louis Encephalitus to the nematode parasite, *Strelkovimermis spiculatus*.

Methods: Egg rafts of *Culex nigripalpus* were collected from the field and hatched in the laboratory. Cultures of the mermithid nematode *Strelkovimermis spiculatus* were flooded with well water and the preparasites obtained were exposed to *Cx. nigripalpus* in the following manner: (1) different ratios of preparasites to larvae (1, 2, 4, 8, and 16:1), (2) different preparasite age (0-7 days) at 5° and 24° C, (3) different host age (0-9 days), and (4) preparasites exposed to larvae and reared at 10, 15, 20, 25, and 30°C. The results were compared with those for *Cx. quinquefusciatus*, which is the host used for mass rearing *S. spiculatus*.

Results: There was no significant difference in percent infection or total nematodes produced when *Cx. nigripalpus* or *Cx. quinquefasciatus* were exposed to increasing numbers of preparasites. This was also true when the two hosts were exposed to preparasites of different ages. The infection level dropped from 80 to 5% as the preparasites aged to 7 days, and preparasites lost their ability to infect after 6.5-7 days as no nematodes were produced. Percent infection and total adults were higher in *Cx. nigripalpus* than in *Cx. quinquefasciatus* when *S. spiculatus* was exposed to the two hosts at different ages. However, there was a decline in responses with larval age. *Culex nigripalpus* and *Cx. quinquefasciatus* responded similarly when infected with the nematode and reared at temperatures from 10° to 30° C. High mortality occurred in both hosts at temperatures below 15° C, but at 30° C, high mortality occurred only in *Cx. quinquefasciatus*. Although *Cx. nigripalpus* survived at this temperature, no nematode infection occurred, indicating that *S. spiculatus* preparasites did not survive to infect at 30° C. Maximum nematode production and minimum larval mortality occurred at 24° C. *Culex nigripalpus*, the vector for St. Louis Encephalitus is a good host for *S. spiculatus,* as a result, *S. spiculatus* has potential as a biocontrol agent for *Cx. nigripalpus*.

CRIS 6615-32000-032-00D

PARASITES OF MOSQUITOES IN NORTH CENTRAL FLORIDA

T. Fukuda, O.R. Willis and D.R. Barnard

Objective: To determine if new parasites are present in the indigenous population of mosquitoes in north central Florida.

Methods: Mosquito larvae were collected from sites within a 100 km radius of Gainesville. Habitats sampled included, tires, artificial containers, treeholes, floodwater and permanent water sites. The larvae collected were examined in the laboratory for gross abnormalities using a dissecting microscope at 16X magnification. Parasites were identified using light and electron microscopy.

Results: During a 2 month period, 28 collections were made from 16 sites. A total of 10,337 larvae were collected and examined. Sixteen species of mosquitoes were collected with Culex quinquefasciatus (3997 larvae in 8 sites) and *Aedes albopictus* (2200 larvae in 13 sites) the most numerous. Parasites were observed in larvae from 12 sites. The most common parasite was the gregarine, *Ascogregarina taiwanensis*, which was found in *Ae. albopictus* in 10 sites. The microsporidium *Amblyospora sp.* and a virus in *Cx. sp.* were observed at one site; also the microsporidium *Culicosporella lunata* in *Cx. piiosis* was observed in another site. The gregarine and microsporidian parasites produce chronic infections, while the virus produced mortality in 3 days.

CRIS: 6615-32000-032-00D

REDUCTION OF HOUSE FLY POPULATIONS IN A CAGED-LAYER HOUSE WITH STRATEGICALLY PLACED COMMERCIAL BAIT STATIONS

J.A. Hogsette and C.J. Geden

Objective: On one test farm, house fly populations in a 152-m long, 100,000-bird caged-layer house had reached excessive levels. In the manure pit, the surface and the color of the support posts could not be seen because of the number of resting flies. In this study, we sought the means to rapidly reduce adult house fly populations with limited use of pesticides so that developing populations of biological control agents in the manure could continue to grow without being adversely affected.

Methods: The previous method of choice would have been larvadex (cyromazine 3% feed-through, Ciba). However, we chose QuikStrike Fly Abatement Strips (nithiazine 1% AI, Sandoz Agro, Inc.). These strips (12 x 47 cm) are coated on both sides with the pesticide formulated with an attractant. Flies feed on the pesticide and die within 15 seconds. In the manure pit, QuikStrike strips were placed ca. 1.5 m high on the downwind side of 40 posts in alternating rows starting at the center of the house and working towards both ends. Flies had to be physically removed from the posts to make room for application of the strips. By the time the last strip was in place, mounds of dead flies had formed at the bases of treated posts. Flies were monitored with spot cards that were changed once each week.

Results: QuikStrike reduced extremely large house fly populations by 91% in less than 4 weeks, thus providing nascent populations of beneficials with a competitive edge over developing larval fly populations. Moreover, QuikStrike was an economically favorable choice, the entire treatment cost $260.00, whereas a 4-week treatment with larvadex costs $1,500.00.

POSTHARVEST

AND

BIOREGULATION

CRIS - 6615-43000-007-00D--Population Management of Insects to Protect Stored Products

CRIS - 6615-43000-008-00D--Detection and Population Estimation of Stored Product Insects

CRIS: 6615-43000-007-00D

THE EFFECTS OF JUVENOID AGONISTS ON THE PHYSIOLOGY OF PLODIA INTERPUNCTELLA

S. Dyby and D.L. Silhacek

Objective: To determine the physiological basis of juvenoid agonist toxicity during embryogenesis and early larval development of the Indian meal moth.

Methods: Freshly laid eggs were collected from a standardized laboratory culture of the Indian meal moth, Plodia interpunctella. The eggs were carefully aged following egg laying in order to isolate previously established developmental events that occur during embryogenesis. These events were monitored by time lapse photography, light microscopy and fluorescent microscopy. Embryonic development in the presence and absence of the hormone agonists were compared in order to identify any lesion(s) that occur in response to a treatment.

Results: When Plodia interpunctella (Lepidoptera: Pyralidae) eggs are treated with juvenile hormone agonists, a distinctive set of embryonic defects follow. Blastokinesis, the tracheal system, and Malphigian tubules are abnormal and the pattern of eye spots change. The midgut remains open dorsally. Yolk cells are more adhesive, ruffle less, and have few lamellipodia during mid-embryogenesis. Long term effects of juvenile hormone agonists lead to molting failures, pink larval pigmentation, supernumerary molts, larval-pupal intermediates, and adults with curly wings.

Several small GTP binding proteins such as Rho, Cdc42 and Rac regulate the organization of F-actin within cells. Rho regulates stress fiber formation, focal adhesions, and have manifold effects on cell shape and metabolism (Ridley and Hall, 1992; Chong et al., 1994; Machesky and Hall, 1996). The changes in fenoxycarb-induced yolk cell behavior during embryogenesis suggested that Cdc42 and/or Rho could be activated or possibly Rac could be inactivated. Lysophosphatidic acid (LPA) or bombesin, a synthetic peptide, activates Rho. Injecting LPA into eggs during the first third of embryogenesis produced abnormal blastokinesis, tracheal system defects, changes in eye spot pattern, and abnormal Malpighian tubules, similar to defects seen when embryos were treated with juvenile hormone agonists. Injecting LPA into larvae caused supernumerary molts, larval-pupal intermediates, molting failures, curly-winged adults, and changes in pigmentation normally associated with responses to juvenile hormone or its agonists. Injections of fenoxycarb or juvenile hormone I produce a similar set of defects, whereas embryos and larvae develop normally after injections of buffers or linolenic acid. This evidence strongly indicates that juvenile hormone action in the insect is mediated, at least in part, by the GTP-binding protein, Rho.

CRIS: 6615-43000-007-00D

INTERACTIONS OF ECDYSTEROID AND JUVENOID AGONISTS ON LARVAE OF *Plodia interpunctella*

H. Oberlander and D.L. Silhacek

Objective: To determine the mutual influence of non-steroidal ecdysteroid agonists and juvenile hormone mimics on the growth and metamorphosis Indianmeal moth larvae.

Methods: The effects of the hormone mimics were assessed by rearing last instar larvae on diet treated with RH-5992 or RH-2485 (Rohm and Haas Co.). Larvae were monitored for effects of the ecdysteroid agonists on weight, metamorphosis and mortality. We also examined the effects of simultaneous treatment with a juvenile hormone mimic, either methoprene or fenoxycarb.

Results: Larvae treated with either of the ecdysteroid agonists at a concentration of 5 ppm or higher gained less weight and had greater mortality than did larvae reared on control diet. For example, the weights of control larvae increased by approximately 400% by day 2, compared with only a 50% increase in weight when the larvae were treated with 25 ppm of RH-2485 or RH-5992. Similarly, mortality in control larvae was less than 10%, but was as much as 90-100% in larvae reared on diet treated with one of the ecdysteroid agonists.

The JH mimics prevented adult emergence, and the larvae continued to feed throughout the month-long observation period. However, larvae treated with a juvenile hormone mimic gained weight despite the presence of an ecdysteroid agonist in the diet. On diets treated with 0.1 ppm of RH-2485 or RH-5992, JH-treated larvae gained even more weight than did untreated controls. Interestingly, although the addition of a JH mimic to ecdysteroid treated diet resulted in increased weight, it did not lead to reduced mortality. In fact, combinations of a JH mimic with 10 ppm RH 2485 or RH 5992 resulted in nearly 100% mortality compared with 40-70% mortality without the JH compounds. These results indicate that JH mimics not only overcame the inhibitory effects of ecdysteroid agonists on weight gain, but they also resulted in increased mortality compared with moderate doses of ecdysteroid agonists alone.Thus, the JH agonists are not simply working antagonistically to the ecdysteroid mimics and countering their effects. First of all, the JH agonists have the unique property of preventing metamorphosis, an effect not demonstrated by the ecdysteroid agonists. Secondly, the extended larval life of test insects treated with JH agonists did not make them immune to the mortality caused by high does of ecdysteroid agonists, and in fact there was the surprising result that moderate doses of ecdysteroid agonist were more lethal when combined with a JH agonist. Since strategies to combat resistance of insects can employ utilization of combinations of chemicals with different modes of action, our results point to the possibility of combining IGRs that mimic both juvenile hormone and ecdysteroids for maximum effectiveness in controlling stored product insects.

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TWO NEW D-E-A-D BOX GENES EXPRESSED IN OVARIES OF THE MOTH, *PLODIA INTERPUNCTELLA*

O.P. Perera and P.D. Shirk

Objective: To identify the genes of germ cell specific proteins in the Indianmeal moth, *Plodia interpunctella*, that can be used to develop molecular genetic methods for production of sterile insects. Homologs for the *Drosophila melanogaster vasa* gene, an RNA helicase which is expressed constitutively through out development in germ cells, were screened for using degenerate PCR primers.

Methods: Degenerate PCR primers to the A- and B-ATP binding motifs of D-E-A-D box proteins were used to screen and isolate RNA helicases from a library of vitellogenic pharate adult ovarian cDNA of *P. interpunctella*.

Results: Clones Piv2-6 and Piv2-17 of the PCR products contained the conserved ATP-A and ATP-B sequences that are common to RNA helicases. Full length cDNA clones isolated from the cDNA library showed these two RNA helicases have considerable regions of similarity with members of the D-E-A-D box protein family and contained most of the consensus motifs present in these proteins. The predicted amino acid sequence of Piv2-6 had 65% similarity with the maternally expressed DEAD5 locus of mouse which is constitutively expressed in germ cells. The predicted amino acid sequence of Piv2-17 had 42% similarity with the Scl9328.3 locus from *Sacromyces cervisiae* and 33% similarity with the ME31 locus in *Drosophila* which is maternally expressed by nurse cells during vitellogenesis. Sequences between the conserved motifs of both RNA helicases were highly variable. Northern analysis of RNA from ovaries, testes, and body walls (fat body, epidermis, muscle, etc.) showed that Piv2-6 was highly expressed in the ovaries and to a lesser amount in the testes were as Piv2-17 was present only in vitellogenic ovaries.

Sequence similarity between the predicted amino acid sequences for *Drosophila vasa* and helicases, Piv2-6 and Piv2-17, from *P. interpunctella*.

```
DMVASA     1 MSDDWDDEPI VDTRGARGGD WSDDEDTAKS FSGEREGDGV GGSGGEGGGY QGGNRDVFGR IGGGRGGGAG GYRGGNRDGG GFHGGRREGE RDFRGGEGGF

DMVASA   101 RGGQGGSRGG QGGSRGGQGG FRGGEGGFRG RLYENEDGDE RRGRLDREER GGERRGRLDR EERGGERGER GDGGFARRRR NEDDINNNNN IAEDVERKRE
Piv2-6       --TLTKGNKS WSSTAVAAAL ELVDPP.C.N SARAYSYHND -HF..ILVGY N-K.LSS.QV S.MANQW.Q. AEELEITN.V AGLGLKKSLD QTAESDDAPD

DMVASA   201 FYIPPEPSND AIEIFSSGIA SGIHFSKYNN IPVKVTGSDV PQPIQHFTSA DLRDIIIDNV NKSGFKIPTP IQKCSIPVIS SG--RDLMAC AQTGSGKTAA
Piv2-6       AAN.A.A.-- ---LLMKI.R Q.LVE..LDI EIQ.KDPNSP LYSVKT.EAL H..PNLLKG. YDM..NA.SK ..ETAL.TLL ADPPQNMI.Q S.S.T.....
Piv2-17      ...-------- I.TV.T---- -------FT. .NMDEEKKVM ------.HEM E.D.R.LKAI SQLSWSE..L ..ATA..LLL E.--..VLMR AR........

DMVASA   301 FLLPILSKLL EDPHELELGR PQVVIVSPTR ELAIQIFNEA RKFAFESY-- LKIGIVYGGT SFRHQNECIT RGCHVVIATP GRLLDFVDRT FITF-EDTRF
Piv2-6        .V.AM....D TSKNY----- ...LCL..TY ..AI.TGEV. A.M.-KFCPE IKLKYAVR.E EVPRGTKI-- -TDHII.G.. .KM..WGVKF GMFDLSKIRV
Piv2-17       .TV.VIQ.VL NLKNTSRHQS IRALVL..S. ..CG..ASVI GDLTLKCARE VRCVDISSSG DMQIQKSLLL DKPDI.VS.. S.V.AHLKAN NLRLK.ELAM

DMVASA   401 VVLDEADRML DMGFSEDMRR IMTHVTMRPE HQTLMFSATF PEEIQRMAGE FLKNYVSVAI GIVGGACSDV KQTIYEVNKY AKRSKLI-EI LSEQADG-TI
Piv2-6       F......V.I .RQGHQ.-QC .RIHKCLPST C.MMF..... DSAVMEFAEI IVP.PIIIRL LREEESLDNI ..YYVKCKSS EEKY.A.CN. YGVITI.QA.
Piv2-17      L.........

DMVASA   501 VFVETKRGAD FLASFLSEKE FPTTSINGDR LQSQREQALR DFKNGSMKVL IATSVASRGL DIKNIKHVIN YDMP------ SKIDDYVHRI GRTGCVGNNG
Piv2-6       I.CH.RKT.G WLSEKM.KDG HSVAVLS.EL TVEQ.IAV.D R.RA.LE... .T.N.L...I .VEQVTL.V. F...MDMNKK ADCET.L... ....RF.KA.

DMVASA   601 RAQSFFDPEK DRAIAADLVK ILEGSGQTVP DFLRTCGAGG DGGYSNQNFG GVDVRGANYV GDATNVEEEE QMD
Piv2-6       I.INLIDS-- --SL.MEICN NI.AHFGKKI KL.DIED.EE IEKIGA
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Abbreviations " " = identity; "upper case letters" = non-identity; and "-" = insertion. Underlined sequences indicate conserved regions with other DEAD box members.

CRIS: 6615-43000-007-00D

A CDNA CLONE OF YP4, A FOLLICULAR EPITHELIUM YOLK PROTEIN SUBUNIT, IN THE MOTH, *PLODIA INTERPUNCTELLA*

O.P. Perera and P.D. Shirk

Objective: To identify genes for follicle cell-specific proteins in the Indianmeal moth, *Plodia interpunctella*, that can be used to develop molecular genetic methods for production of sterile insects. The gene for YP4, a subunit of the follicular epithelium yolk protein, was isolated because it is highly expressed during vitellogenesis and has been shown to be regulated in part by the level of the ecdysone hormone.

Methods: Partial cDNA clones for YP4 were isolated from a pharate adult female ovarian cDNA expression library in Lambda Zap II by using a degenerate PCR primer designed on the basis of the N-terminal amino acid sequence for mature YP4 and the T7 primer from the Lambda Zap II vector. The 5' sequence of the YP4 transcript was determined by 5' RACE PCR of ovarian mRNA using YP2 sequence-specific nested primers. Northern blot analysis provided an estimate of the size of the mature transcript in the ovaries during vitellogenesis.

Results: The cDNA clones for YP4 were initially screening for using antigen selected YP4 antiserum, but none were identified after screening 10^6 recombinant phage. In order to identify the cDNA clones, a degenerate PCR primer was designed to six amino acid residues identified in the N-terminal sequence for mature YP4. The N-terminal sequence of YP4 was determined to be RIDVQLSGEFNDDSHNNLKVYYSGSQASVI and the PCR primer was based on the GEFNDD residues. The reverse PCR primer was the T7 primer found in the Lambda Zap II vector. The PCR product was cloned into the *pCRII* vector and sequenced. The 5' region of the YP4 transcript was determine by 5' RACE PCR based on nested primers designed from the YP4 cDNA sequence. The cDNA and 5' RACE sequencing showed the YP4 transcript was 991 bp in length with a single open reading frame for a predicted polypeptide of 299 amino acids. Northern analysis showed a single YP4 transcript was present in ovarian RNA that was approximately 1 kb in length. The predicted amino acid sequence for YP4 from *P. interpunctella* was most closely related to the YP4 gene from *Galleria mellonella* and the spherulin 2a protein from the slime mold, *Physarum polycephalum*.

Similarity between the predicted amino acid sequence for the YP4 subunit of *P. interpunctella* and related proteins. Significant sequence similarity was shared with only two known genes: YP4 from *Galleria mellonella* and spherulin 2A from (Abbreviations: . = identical residues; - = insertions; GmYP = YP4 from *G. mellonella*; PiYP4 = YP4 from *P. interpunctella*)

```
            1                                                                                                   100
     PiYP4  MASLYLLLVL PALVL.ARID VQLSGEFNDD SHNNLKVYYS GSQAGVITDA ERTTFGLSDA ILKDSIGAYF GRRPDDAYLR SPTPWG.... .DLYSTFGWD
     GmYP4  -v-K--ail- FiPiiY-k-Q -nvVaSE-eA ETGEP....G WKnvDi---N --Y--Q-t-N N--NAvQS-- -Q-----f-- ------.... .---QVy--P
Spherulin 2a                  MAFQ -naHvG...N RTASSh.... -VVERiM--S d-RA---DGD N-FRAvERfR --W-TGawv- --AIA-GVDL YQa-AHQ--R

            101                                                                                                 200
     PiYP4  QVLRTLVPKN GKILGITSQP MIITKQLFEN NSSKPATFDV GISQSVQNTV KSSWSQGGSL NVAQEINYGF NIEVIKGGGK TAFSYTSTWG RNTEKSESVT
     GmYP4  --ars-SSSE S-Y-Qvs-K- S--LT-H-R- --tQ----Ka Q-Q-Q----- T-t-EK--E- T-g---E--- D-K-vSv--- -S-----R-- ESVS---t--
Spherulin 2a  --vTR-EPIS ST-HHPNtDR TTvVTARLS- ---F-gE-Fa Nl-nETT-sa Tt---STHGi E-g-SvS-.. S-G-vS..-E -S-G-sYQ-- -GG-QtTASs

            201                                                                                                 300
     PiYP4  IGSQSGMKIL LQPGQAVVAQ LQATKGTIRM EVEYEASLSG ASAVNYDDGY RGHHFWSLDI RAIMASVGLP NQKVSKEVIE IDFYSQSRVV VNDKSTNIKL
     GmYP4  v--E--VE-T -E----ii-E -L--r--MEI Q-d---t--- -t----ANTf k---HfWaSg hQCcDD-WRL EPhR-..... lPGSY-A-ll lqLSRCDQrC
Spherulin 2a  vSFVt-VTvH -----g-ivR -L-EQ-Wa-I TTR-R---t- HV-Q-fNPPH QG----AHSv NS-LQAS--- T-IFIENTvd vG-fAn-h-D Mq-LV-GvIv

            301                                     342
     PiYP4  MEIGF
     GmYP4
Spherulin 2a  PIgTDKIFRP LALKYDEIKE DDEKFADEKE QQKGEHKKET KH
```

CRIS: 6615-43000-007-00D

YP2, A FOLLICULAR EPITHELIUM YOLK PROTEIN SUBUNIT, HAS AN UNUSUAL INSERT IN THE 5' CODING REGION OF THE CDNA IN THE MOTH, *PLODIA INTERPUNCTELLA*

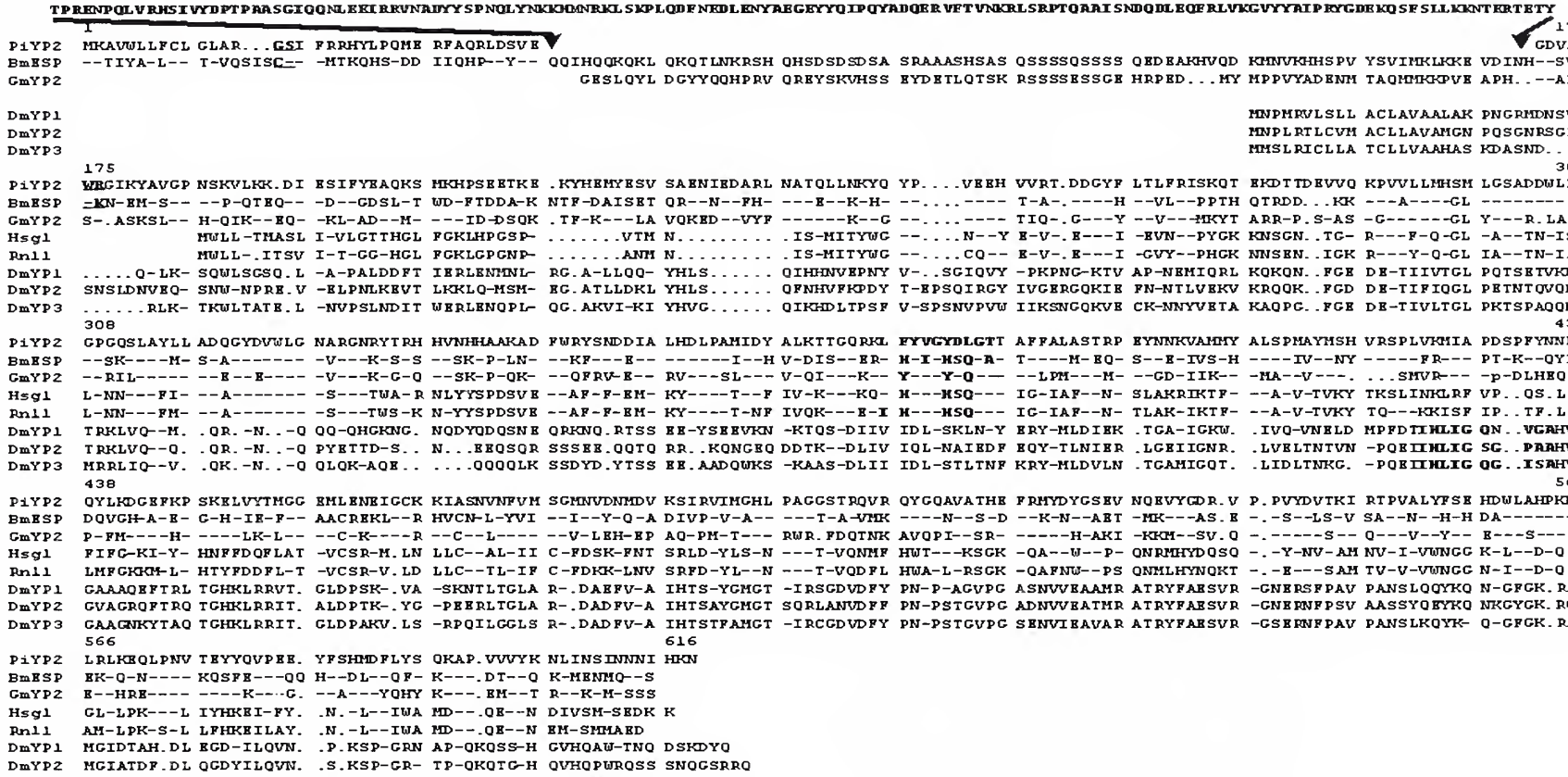
P.D. Shirk and O.P. Perera

Objective: To identify genes for follicle cell-specific proteins in the Indianmeal moth, *Plodia interpunctella*, that can be used to develop molecular genetic methods for production of sterile insects. The gene for YP2, a subunit of the follicular epithelium yolk protein, was isolated because it is highly expressed during vitellogenesis and has been shown to be regulated in part by the level of the ecdysone hormone.

Methods: Partial cDNA clones for YP2 were isolated from a pharate adult female ovarian cDNA expression library in Lambda Zap II by screening with antigen selected YP2 antiserum. The 5' sequence of the YP2 transcript was determined by 5' RACE PCR of ovarian mRNA using YP2 sequence-specific nested primers. Northern blot analysis provided an estimate of the size of the mature transcript in the ovaries during vitellogenesis.

Results: The six partial YP2 cDNA clones were isolated by screening 10^3 recombinant phage using the antigen selected YP2 antiserum. The identity of the YP2 cDNA clones was confirmed by comparing the predicted N-terminal amino acid sequence with the first 30 amino acid residues identified in the mature YP2 which were determined to be SIFRRHYLPQMERFAQRLDSVETPRENPQL. The 5' region of the YP2 transcript was determined by 5' RACE PCR using nested primers designed from the YP2 cDNA sequence. The cDNA and 5' RACE sequencing showed the YP2 transcript was 1982 bp in length with a single open reading frame for a predicted polypeptide of 616 amino acids. Northern analysis showed a single YP2 transcript was present in ovarian RNA that was approximately 2 kb in length. The predicted amino acid sequence for YP2 from *P. interpunctella* was most closely related to egg specific protein (ESP) from *Bombyx mori* and the partial YP2 sequence from *Galleria mellonella*. YP2 from *P. interpunctella* also showed similarity with vertebrate lipases and contains a conserved lipid binding region. However, the 5' coding region of YP2 from *P. interpunctella* contained an insert of approximately 438 bp that had replaced an approximately 270 bp region as compared with ESP from *B. mori* and YP2 of *G. mellonella*. This suggests that the insert occurred by a recombinational event internal to the YP2 structural gene of *P. interpunctella*.

Similarity between the predicted amino acid sequence for the YP2 subunit of *P. interpunctella* and related proteins from Lepidoptera, Diptera, and vertebrate lipases. The Lepidoptera are represented by *Bombyx mori* egg specific protein (BmESP) and the partial *Galleria mellonella* YP2 (GmYP2); the Diptera by *Drosophila melanogaster* yolk polypeptides 1-3 (DmYP1-3); and the vertebrate lipases by human gastric lipase (Hsgl) and rat lingual lipase (Rnll). The sequence positioned by arrows shows the recombinational insert in YP2. (Abbreviations: . = identical residues; - = insertions.)



CRIS: 6615-43000-007-00D

THE POTENTIAL OF INSECT GROWTH REGULATOR APPLICATIONS FOR MANAGING MOTH POPULATIONS IN PACKAGED COMMODITIES

D.L. Silhacek and C. Murphy

Objective: To determine if treatments with juvenoid agonists (JHAg), ecdysteroid agonists or chitin synthesis inhibitors can effectively protect commodities from insect damage during storage.

Methods: We previously reported the results of laboratory tests on the Indian meal moth, Plodia interpunctella, that showed adult females laid non-viable eggs following a six-hour or longer exposure to surfaces treated with a JHAg. In our studies during the past year, a number of methods for delivering JHAg to Indian meal moths and several species of beetles that infest stored commodities were studied.

To more closely simulate warehouse storage conditions, we scaled-up to one cubic meter by fabricating closed cylinders from corrugated cardboard. The JHAg, fenoxycarb, was applied to all inner surfaces of the paper cylinders. Five Plodia mating pairs were introduced into each cylinder that also contained a boxed (3600 cm3) commodity. The boxed commodity was covered with a fenoxycarb-treated paper over-wrap that excluded adult moths but not crawling larvae. In some tests, the JHAG was synergized with 0.1% piperonyl butoxide.

The effects of IGRs on the development and reproduction of the red flour beetle, Tribolium castaneum, the saw-toothed grain beetle, Oryzaephilus surinamensis, and the lesser grain borer, Graminella sonorus, were examined in laboratory tests. The JHAG, fenoxycarb and pyriproxyfen, the CSI, chlorfluazuron, and the ecdysteroid agonist (EAg), RH-5992, were tested by having adults walk on IGR-treated paper, by incorporating IGR into the adult diet or by topically applying to eggs or to adult females. For each treatment, we examined egg viability and mortality during subsequent development. When appropriate, the number of eggs laid was also monitored.

Results: Tests using the 3600 cm3 boxes showed that the hatchability of eggs was less than 10% when laid by Plodia females kept in continuous contact with paper treated with 2ug/cm2 of fenoxycarb. In the closed cylinder experiments, the results indicated that insect infestation of the boxed commodity could be prevented when the surfaces were treated with 8.0 ug of JHAg/cm2. Adding any one of the synergists piperonyl butoxide, triphenyl phosphate, or diethyl maleate to the JHAG-treated surface did not improve the effectiveness of the treatment. Likewise, elevating the test temperature from 26 C to 34 C had little effect on hatchability.

All of the beetles tested produced nonviable eggs when the adults were feeding on a JHAG diet (>1 ppm). However, the beetles were able to recover rapidly (within eight hours) and lay fertile eggs when removed from the treated diet. In the red flour beetle, JHAg were totally effective in preventing egg hatch when topically applied to the egg by dunking or when adult females were placed on treated diet (2ppm or higher) and eggs were laid. The inclusion of synergists was ineffective.

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MONITORING INSECT INFESTATION OF STORED GRAIN BY TRAPPING AND SPATIAL ANALYSIS

R.T. Arbogast, P.E. Kendra, D.K. Weaver and D. Shuman

Objective: Monitoring bulk storages of grain to detect and estimate levels of insect infestation remains problematic. Sampling grain or deploying and reading traps requires repeated bin entry, which can be hazardous and is costly in terms of time expended. Sampling can provide an adequate estimate of infestation level (insect population density) if enough samples are taken, but sampling requires additional time to separate insects from grain and does not detect infestation as early as trapping. Trapping provides a sensitive method of detection, but available methods for interpreting trap catch, either as population density or as a risk factor, are inadequate. Our objective was to field test EGPIC (Electronic Grain Probe Insect Counter), a system that automatically records the numbers of insects captured in pitfall traps. Trap interpretation will be addressed in later studies.

Methods: The EGPIC system was described by Shuman, Coffelt and Weaver (1996. Trans. Am. Assoc. Agric. Eng. 39:1773-1780). Our field test was done at a seed processing plant in Williston, Florida on 68 t of wheat stored in a metal bin 5.6 m in diameter X 5.6 m high. The grain was placed in storage on or about July 24, 1997 and removal began on October 21. Eight EGPIC traps were deployed in the surface layer of the grain: 0.9 m from the center at north, east, south and west; and 1.8 m from the center at northeast, southeast, southwest and northwest. Insects were recovered from these traps at weekly intervals for 6 of the 21 weeks the grain was in storage and placed in alcohol for later identification and counting in the laboratory. Grain samples were taken at each trap site for moisture determination (Motomco Model 919 moisture tester) and grain temperature was recorded at each site with Omega temperature loggers (RD-TEMP-XT). Contour analysis of the counts was done with surfer 6.02 (Golden Software).

Results: The test revealed several problems with the EGPIC system that will require correction before it can be tested further in the field. Because of these problems, no electronic insect counts were obtained for comparison with the manual counts. Manual counts for the first and second weeks of storage showed that the insect population was concentrated in the southwest quadrant of the bin with a maximum west-southwest of the center (Fig. 1). *Cryptolestes pusillus* was the dominant species, followed by *Carpophilus dimidiatus*, *Ahasverus advena*, *Typhaea stercorea*, *Sitophilus oryzae*, *Cryptolestes ferrugineus*, *Sitophilus zeamais* and small numbers of various other species (Fig.2). Trap catches were also obtained for weeks 7-10, but processing and analysis has not been completed. Mean (±sd) grain moisture content for the bin varied from 11.6% (±0.1) to 11.9% (±0.1) during storage. Maximum moisture content declined from 12.2% shortly after storage to 11.7% at the time of removal. The higher initial readings were for grain samples taken from the southeastern, southern and southwestern portions of the bin.

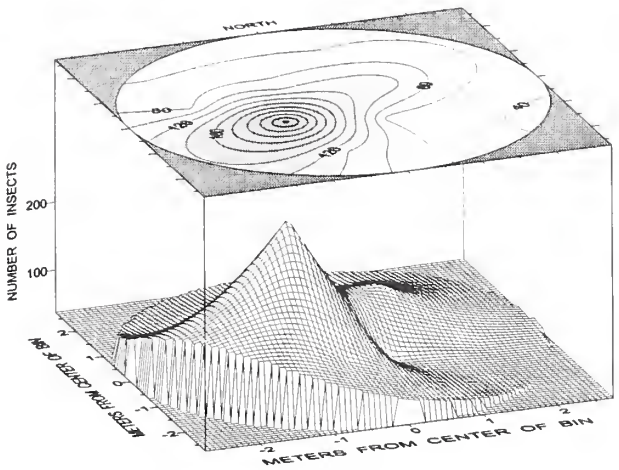

Figure 1. Spatial distribution of the insect population in a bin of stored wheat at Williston, Florida after two weeks of storage.

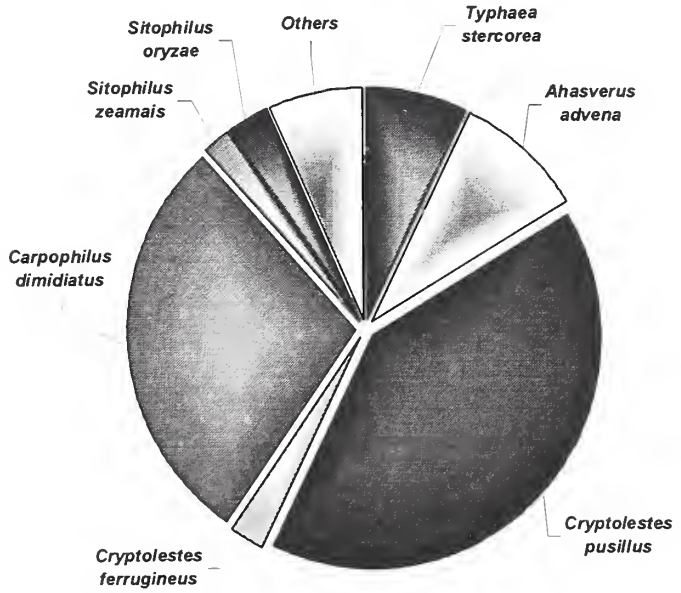

Figure 2. Species composition of the insect population in a bin of stored wheat at Williston, Florida after one week of storage.

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MONITORING INDIANMEAL MOTHS IN RETAIL STORES BY TRAPPING AND SPATIAL ANALYSIS

R.T. Arbogast and R.W. Mankin

Objective: Better methods of monitoring are needed to facilitate integrated management of storage pests in retail stores. Such methods should be capable of detecting insect infestations quickly and locating foci of infestation. The methods should be inexpensive and easy to apply, and they must be inconspicuous. Our objective was to develop methods that meet these requirements for monitoring the Indianmeal moth, *Plodia interpunctella* (Hübner) in large retail stores.

Methods: Pheromone-baited sticky traps and contour analysis of trap counts were used to map the spatial distribution of Indianmeal moths in three discount department stores. The traps (Agrisense S. P. Locator traps) were small (7 X 10 X 1.5 cm.) and were easily concealed on the underside of shelves, to which they were attached by velcro. The pheromone dispensers were rubber septa (Agrisense Minimoth pheromone dispensers) designed to attract over short distances. The major pheromone component is Zeta and is emitted at a mean rate of 2.40 ng./septum/hr. Short range traps are preferred for spatial analysis, because they provide better resolution of an insect population into local components and thus provide a sharper focus on spatial pattern. The traps were distributed throughout the stores with more in the pet food and grocery sections and fewer in sections such as hardware and clothing. Trap locations were recorded using a rectangular coordinate system with the origin at one corner of the store. Trapped moths were counted one hour after the traps were deployed and again after 4, 24, 48, 72 and 96 hours. Contour analysis was done with Surfer 6.02 (Golden Software).

Results: Indianmeal moths were detected in all three stores, but the level of infestation varied. Store 1 showed high trap catches in the pet supply area, probably produced on spillage left under the bottom shelves. The largest focus of infestation was apparent after one hour, and although the number of moths trapped continued to increase for 96 hours, the distribution did not change significantly after 24. The pet supply area of Store 2 had been cleaned shortly before we began trapping. No moths were captured during the first hour and only one during the first four. After 96 hours, three moths had been captured in pet supplies and four in other parts of the store, but no trap captured more than one moth. Store 3 showed moderate trap catches with one focus, already apparent after one hour, in the pet supply area. Although the number of moths trapped continued to increase, the spatial pattern remained unchanged over the rest of the trapping period. Interpretation of trap catch remains a problem in many trapping applications. In retail stores, we want to detect infestation and locate the foci (or sources) so we can correct the problem by removal of infested products or other measures. We are also interested in assessing the effectiveness of treatment by trapping again following treatment. Spatial analysis of trap counts serves both purposes, and no other interpretation is required. Our results suggest that one to 24 hours of trapping with a sufficient number of well-distributed, short-range pheromone traps is enough to detect infestations of Indianmeal moths and locate foci of infestation.

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ACOUSTIC DETECTION OF INSECT PEST POPULATIONS IN SOIL AND WOOD

R.W. Mankin and D. Shuman

Objective: *Phyllophaga* and *Diaprepes* beetle grubs are important pests in many different agricultural environments, including turf, pastures, and citrus groves. Termites are important pests in urban environments. All of these insects are difficult to detect and monitor because they remain subterranean for most of their life cycle. However, there is an urgent need for nondestructive integrated pest management tools to help estimate population levels, distribution, and economic damage thresholds. It may be possible to adapt acoustic technology to detect these insects and estimate their population levels.

Methods: Different acoustic and vibration sensors were tested in laboratory and field environments to determine the feasibility of developing a portable detection system that could be adapted for use by nontechnical personnel. Piezoelectric sensors were connected directly to a high-gain, portable digital tape recorder (DAT). The recorded signals could be monitored on site by an observer with headphones or analyzed in the laboratory using digital signal processing software. A more expensive, but technically precise method was to connect an accelerometer through a portable amplifier to the DAT-headphone system. Several different sensor placements were tested, including attachment to stakes inserted in the soil, and attachment to tree trunks. Outdoor experiments included high and low background noise and wind conditions.

Results: The key to successful use of acoustic technology for monitoring hidden infestations in soil or wood is increasing the signal to noise ratio in the frequency range between 400 and 800 Hz. This range lies above the range of greatest background noise energy, and captures a significant portion of feeding and movement sounds by insects near the sensor. These low-frequency signals also carry over longer distances in soil than high-frequency signals. Accelerometers work better in this frequency range than piezoelectric sensors. In the laboratory, the accelerometers work best for detecting *Diaprepes* grubs feeding on roots when they are attached to a tree trunk. Outdoors, however, the signal to noise ratio of the accelerometers is improved when they are attached to 15-20-cm nails rather to tree trunks because wind induces broadband noise in the trunks that masks grub sounds. *Phyllophaga* grubs also could be most easily detected above background by the use of accelerometers attached to nails that extended to their 15-20-cm feeding depth. Termites could be detected by an accelerometer attached to a nail inserted anywhere on a 200-cm-long plank in a low-noise, unshielded laboratory.

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DETECTION AND POPULATION ESTIMATION OF STORED PRODUCT INSECTS

D. Shuman, R.W. Mankin, R.T. Arbogast and D.K. Weaver

Objective: To develop and evaluate automated systems for quantifying hidden infestations in grain samples and for monitoring infestations in stored-products.

Methods: a)The Acoustic Location Fingerprinting Insect Detector (ALFID) System for grain sample inspection had displayed a susceptibility to building vibrations that increased grain settling sounds and thereby degraded performance during a field test at a GISPA inspection office at a New Orleans grain elevator. The frame holding the ALFID grain container and its surrounding sound attenuation box were both redesigned to address this problem.

b)The software for the EGPIC System, used for automated monitoring of infestations using electronic grain probes, was modified to accommodate unexpected field conditions at a commercial facility in Williston, FL.

c)The design of printed circuit boards for The SMARTS Network for transmitting data in a large-scale automated infestation monitoring system has been undertaken for purposes of conducting a system field test.

d)To develop a new design for an acoustic infestation monitoring system suitable for large-scale applications, insect acoustic data were digitally recorded and then analyzed by custom designed software to determine the detection range and directionality of piezoelectric disk sensors.

e)Commercial insect electrocuters were modified to explore their usage for monitoring flying stored-product insects.

Results: a)A new ALFID frame was constructed incorporating pneumatic vibration dampers and pneumatic wheels. The new frame also improves the portability of the 300+ lb. unit by permitting it to be moved into a van and through standard width doorways without disassembly. A ramp and a floating platform were built for a laboratory simulation of a vibrating floor. In addition, with the awarding of an SBIR grant to DRT, Inc., a CRADA was extended and 16 fluidic sensors for a fluidic implementation of ALFID were constructed and tested in our laboratory.

b)The new software can now recover from and document an unlimited number of power outages while performing its monitoring function. It can also read any size data file created during its acquisition of insect counts and system events. The EGPIC System was awarded a patent on July 8, 1997 by the U.S. Patent and Trademark Office.

c)Design criteria for the SMARTS Network printed circuit boards have been established and the board layout is underway. The SMARTS patent application was declared allowable by the U.S. Patent and Trademark Office.

d)The piezoelectric disk sensor range was found to be greater than 50 cm. This information indicates the size of grain containers needed to conduct acoustic tests with variable insect densities. Such tests will permit computer simulations of different possible hardware signal analysis designs to find an optimal configuration for estimating insect populations in grain.

e)The modified insect electrocuters were able to electronically count the number of moths that entered them. A patent disclosure of this method of insect monitoring was approved for patenting by an ARS committee.

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